

SUBSTITUTED 6-CYCLOHEXYLALKYL SUBSTITUTED 2-QUINOLINONES
AND 2-QUINOXALINONES AS POLY(ADP-RIBOSE) POLYMERASE
INHIBITORS

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Field of the invention

The present invention relates to inhibitors of PARP and provides compounds and compositions containing the disclosed compounds. Moreover, the present invention provides methods of using the disclosed PARP inhibitors for instance as a medicine.

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Background of the invention

The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) is a member of the PARP enzyme family consisting of PARP-1 and several recently identified novel poly(ADP-ribosylating) enzymes. PARP is also referred to as poly(adenosine 5'-diphospho-ribose) polymerase or PARS (poly(ADP-ribose) synthetase).

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PARP-1 is a major nuclear protein of 116 kDa consisting of three domains : the N-terminal DNA binding domain containing two zinc fingers, the automodification domain and the C-terminal catalytic domain. It is present in almost all eukaryotes. The enzyme synthesizes poly(ADP-ribose), a branched polymer that can consist of over 200 ADP-ribose units. The protein acceptors of poly(ADP-ribose) are directly or indirectly involved in maintaining DNA integrity. They include histones, topoisomerases, DNA and RNA polymerases, DNA ligases, and Ca^{2+} - and Mg^{2+} -dependent endonucleases. PARP protein is expressed at a high level in many tissues, most notably in the immune system, heart, brain and germ-line cells. Under normal physiological conditions, there is minimal PARP activity. However, DNA damage causes an immediate activation of PARP by up to 500-fold.

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Among the many functions attributed to PARP, and especially PARP-1, is its major role in facilitating DNA repair by ADP-ribosylation and therefore co-ordinating a number of DNA repair proteins. As a result of PARP activation, NAD^+ levels significantly decline. Extensive PARP activation leads to severe depletion of NAD^+ in cells suffering from massive DNA damage. The short half-life of poly(ADP-ribose) results in a rapid turnover rate. Once poly(ADP-ribose) is formed, it is quickly degraded by the constitutively active poly(ADP-ribose) glycohydrolase (PARG), together with phosphodiesterase and (ADP-ribose) protein lyase. PARP and PARG form a cycle that converts a large amount of NAD^+ to ADP-ribose. In less than an

hour, over-stimulation of PARP can cause a drop of NAD^+ and ATP to less than 20% of the normal level. Such a scenario is especially detrimental during ischaemia when deprivation of oxygen has already drastically compromised cellular energy output.

Subsequent free radical production during reperfusion is assumed to be a major cause of tissue damage. Part of the ATP drop, which is typical in many organs during ischaemia and reperfusion, could be linked to NAD^+ depletion due to poly(ADP-ribose) turnover. Thus, PARP or PARG inhibition is expected to preserve the cellular energy level thereby potentiating the survival of ischaemic tissues after insult.

Poly(ADP-ribose) synthesis is also involved in the induced expression of a number of genes essential for inflammatory response. PARP inhibitors suppress production of inducible nitric oxide synthase (iNOS) in macrophages, P-type selectin and intercellular adhesion molecule-1 (ICAM- 1) in endothelial cells. Such activity underlies the strong anti-inflammation effects exhibited by PARP inhibitors. PARP inhibition is able to reduce necrosis by preventing translocation and infiltration of neutrophils to the injured tissues.

PARP is activated by damaged DNA fragments and, once activated, catalyzes the attachment of up to 100 ADP-ribose units to a variety of nuclear proteins, including histones and PARP itself. During major cellular stresses the extensive activation of PARP can rapidly lead to cell damage or death through depletion of energy stores. As four molecules of ATP are consumed for every molecule of NAD^+ regenerated, NAD^+ is depleted by massive PARP activation, in the efforts to re-synthesize NAD^+ , ATP may also become depleted.

It has been reported that PARP activation plays a key role in both NMDA- and NO-induced neurotoxicity. This has been demonstrated in cortical cultures and in hippocampal slices wherein prevention of toxicity is directly correlated to PARP inhibition potency. The potential role of PARP inhibitors in treating neurodegenerative diseases and head trauma has thus been recognized even if the exact mechanism of action has not yet been elucidated.

Similarly, it has been demonstrated that single injections of PARP inhibitors have reduced the infarct size caused by ischemia and reperfusion of the heart or skeletal muscle in rabbits. In these studies, a single injection of 3-amino-benzamide (10 mg/kg), either one minute before occlusion or one minute before reperfusion, caused similar reductions in infarct size in the heart (32-42%) while 1,5- dihydroxyisoquinoline

(1 mg/kg), another PARP inhibitor, reduced infarct size by a comparable degree (38-48%) These results make it reasonable to assume that PARP inhibitors could salvage previously ischaemic heart or reperfusion injury of skeletal muscle tissue.

5 PARP activation can also be used as a measure of damage following neurotoxic insults resulting from exposure to any of the following inducers like glutamate (via NMDA receptor stimulation), reactive oxygen intermediates, amyloid β -protein, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its active metabolite N-methyl-4-phenylpyridine (MPP^+), which participate in pathological conditions such as stroke,
10 Alzheimer's disease and Parkinson's disease. Other studies have continued to explore the role of PARP activation in cerebellar granule cells in vitro and in MPTP neurotoxicity. Excessive neural exposure to glutamate, which serves as the predominate central nervous system neurotransmitter and acts upon the N-methyl D-aspartate (NMDA) receptors and other subtype receptors, most often occurs as a result of stroke
15 or other neurodegenerative processes. Oxygen deprived neurons release glutamate in great quantities during ischaemic brain insult such as during a stroke or heart attack. This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors, which open ion channels and permit uncontrolled ion flow (e.g., Ca^{2+} and Na^+ into the cells and K^+ out
20 of the cells) leading to overstimulation of the neurons. The over-stimulated neurons secrete more glutamate, creating a feedback loop or domino effect which ultimately results in cell damage or death via the production of proteases, lipases and free radicals. Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions including epilepsy, stroke, Alzheimer's disease, Parkinson's
25 disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease, schizophrenia, chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Glutamate exposure and stimulation has also been implicated as a basis for compulsive disorders, particularly drug dependence. Evidence includes findings in many animal species, as well as in cerebral cortical cultures treated
30 with glutamate or NMDA, that glutamate receptor antagonists (i.e., compounds which block glutamate from binding to or activating its receptor) block neural damage following vascular stroke. Attempts to prevent excitotoxicity by blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind and hence finding an effective mix of
35 antagonists or universal antagonist to prevent binding of glutamate to all of the receptor and allow testing of this theory, has been difficult. Moreover, many of the compositions

that are effective in blocking the receptors are also toxic to animals. As such, there is presently no known effective treatment for glutamate abnormalities.

The stimulation of NMDA receptors by glutamate, for example, activates the enzyme neuronal nitric oxide synthase (nNOS), leading to the formation of nitric oxide (NO),

5 which also mediates neurotoxicity. NMDA neurotoxicity may be prevented by treatment with nitric oxide synthase (NOS) inhibitors or through targeted genetic disruption of nNOS in vitro.

10 Another use for PARP inhibitors is the treatment of peripheral nerve injuries, and the resultant pathological pain syndrome known as neuropathic pain, such as that induced by chronic constriction injury (CCI) of the common sciatic nerve and in which transsynaptic alteration of spinal cord dorsal horn characterized by hyperchromatosis of cytoplasm and nucleoplasm (so-called "dark" neurons) occurs.

15 Evidence also exists that PARP inhibitors are useful for treating inflammatory bowel disorders, such as colitis. Specifically, colitis was induced in rats by intraluminal administration of the hapten trinitrobenzene sulfonic acid in 50% ethanol. Treated rats received 3-aminobenzamide, a specific inhibitor of PARP activity. Inhibition of PARP activity reduced the inflammatory response and restored the morphology and the
20 energetic status of the distal colon.

Further evidence suggests that PARP inhibitors are useful for treating arthritis. Further, PARP inhibitors appear to be useful for treating diabetes. PARP inhibitors have been shown to be useful for treating endotoxic shock or septic shock.
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PARP inhibitors have also been used to extend the lifespan and proliferative capacity of cells including treatment of diseases such as skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, muscular dystrophy, degenerative diseases of skeletal muscle involving replicative senescence, age-related muscular degeneration,
30 immune senescence, AIDS, and other immune senescence disease; and to alter gene expression of senescent cells.

It is also known that PARP inhibitors, such as 3-amino benzamide, affect overall DNA repair in response, for example, to hydrogen peroxide or ionizing radiation.
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The pivotal role of PARP in the repair of DNA strand breaks is well established, especially when caused directly by ionizing radiation or, indirectly after enzymatic

repair of DNA lesions induced by methylating agents, topoisomerases I inhibitors and other chemotherapeutic agents as cisplatin and bleomycin. A variety of studies using “knockout” mice, trans-dominant inhibition models (over-expression of the DNA-binding domain), antisense and small molecular weight inhibitors have demonstrated the role of PARP in repair and cell survival after induction of DNA damage. The inhibition of PARP enzymatic activity should lead to an enhanced sensitivity of the tumor cells towards DNA damaging treatments.

PARP inhibitors have been reported to be effective in radiosensitizing (hypoxic) tumor cells and effective in preventing tumor cells from recovering from potentially lethal and sublethal damage of DNA after radiation therapy, presumably by their ability to prevent DNA strand break rejoining and by affecting several DNA damage signaling pathways.

PARP inhibitors have been used to treat cancer. In addition, U.S. Patent No.5,177,075 discusses several isoquinolines used for enhancing the lethal effects of ionizing radiation or chemotherapeutic agents on tumor cells. Weltin et al., "Effect of 6(5 - Phenanthridinone, an Inhibitor of Poly(ADP-ribose) Polymerase, on Cultured Tumor Cells", *Oncol. Res.*, 6:9, 399-403 (1994), discusses the inhibition of PARP activity, reduced proliferation of tumor cells, and a marked synergistic effect when tumor cells are co- treated with an alkylating drug.

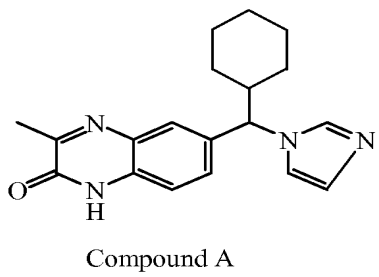
A recent comprehensive review of the state of the art has been published by Li and Zhang in *IDrugs* 2001, 4(7): 804-812.

There continues to be a need for effective and potent PARP inhibitors, and more particularly PARP-1 inhibitors which produce minimal side effects. The present invention provides compounds, compositions for, and methods of, inhibiting PARP activity for treating cancer and/or preventing cellular, tissue and/or organ damage resulting from cell damage or death due to, for example, necrosis or apoptosis. The compounds and compositions of the present invention are especially useful in enhancing the effectiveness of chemotherapy and radiotherapy where a primary effect of the treatment is that of causing DNA damage in the targeted cells.

Background prior art

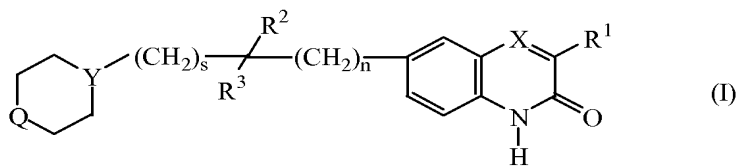
EP 371564, published on June 6, 1990, discloses (1*H*-azol-1-ylmethyl) substituted quinoline, quinazoline or quinoxaline derivatives. The described compounds

suppress the plasma elimination of retinoic acids. More in particular 6-(cyclohexyl-1*H*-imidazol-1-ylmethyl)-3-methyl-2(1*H*)-quinoxalinone (Compound A) is disclosed.



Description of the invention

This invention concerns compounds of formula (I)



the *N*-oxide forms, the addition salts and the stereo-chemically isomeric forms thereof, wherein

n is 0 or 1;

s is 0 or 1;

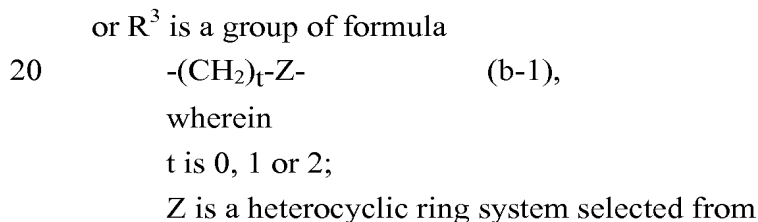
X is $-N=$ or $-CR^4=$, wherein R^4 is hydrogen or taken together with R^1 may form a bivalent radical of formula $-CH=CH-CH=CH-$;

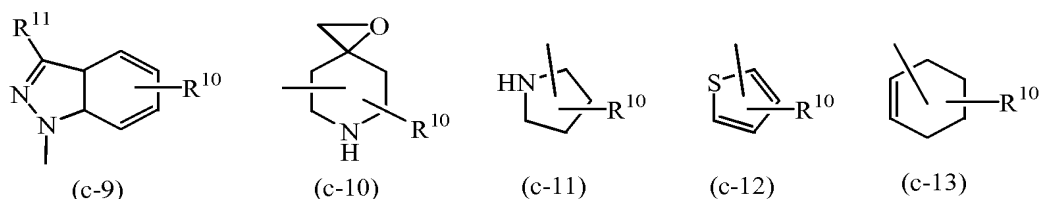
Y is $-N<$ or $-CH<$;

Q is $-NH-$, $-O-$, $-C(O)-$, $-CH_2-CH_2-$ or $-CHR^5-$,

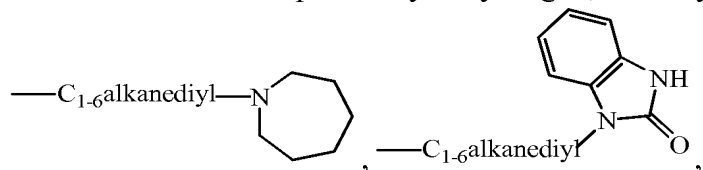
wherein R^5 is hydrogen, hydroxy, C_{1-6} alkyl, aryl C_{1-6} alkyl, C_{1-6} alkyloxycarbonyl, C_{1-6} alkyloxy C_{1-6} alkylamino or haloindazolyl;

R^1 is C_{1-6} alkyl or thienyl;





wherein each R¹⁰ independently is hydrogen, C₁₋₆alkyl, aminocarbonyl, hydroxy,



C₁₋₆alkyloxyC₁₋₆alkyl, C₁₋₆alkyloxyC₁₋₆alkylamino, di(phenylC₂₋₆alkenyl),
 5 piperidinylC₁₋₆alkyl, C₃₋₁₀cycloalkyl, C₃₋₁₀cycloalkylC₁₋₆alkyl,
 aryloxy(hydroxy)C₁₋₆alkyl, haloindazolyl, arylC₁₋₆alkyl, arylC₂₋₆alkenyl,
 morpholino, C₁₋₆alkylimidazolyl, or pyridinylC₁₋₆alkylamino;
 each R¹¹ independently is hydrogen, hydroxy, piperidinyl or aryl;

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aryl is phenyl or phenyl substituted with halo, C₁₋₆alkyl or C₁₋₆alkyloxy

with the proviso that 6-(cyclohexyl-1*H*-imidazol-1-ylmethyl)-3-methyl-2(1*H*)-
 quinoxalinone is not included.

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Whenever the heterocyclic ring system Z contains a —CH₂—, —CH=, or —NH— moiety the
 substituents R¹⁰ and R¹¹ or the rest of the molecule can be attached to the carbon or
 nitrogen atom in which case one or both hydrogen atoms are replaced.

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The compounds of formula (I) may also exist in their tautomeric forms. Such forms
 although not explicitly indicated in the above formula are intended to be included within
 the scope of the present invention.

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A number of terms used in the foregoing definitions and hereinafter are explained
 hereunder. These terms are sometimes used as such or in composite terms.

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As used in the foregoing definitions and hereinafter, halo is generic to fluoro, chloro,
 bromo and iodo; C₁₋₆alkyl defines straight and branched chain saturated hydrocarbon
 radicals having from 1 to 6 carbon atoms such as, e.g. methyl, ethyl, propyl, butyl,
 pentyl, hexyl, 1-methylethyl, 2-methylpropyl, 2-methyl-butyl, 2-methylpentyl and the
 like; C₁₋₆alkanediyloxy defines bivalent straight and branched chained saturated

hydrocarbon radicals having from 1 to 6 carbon atoms such as, for example, methylene, 1,2-ethanediyl, 1,3-propanediyl, 1,4-butanediyl, 1,5-pentanediy, 1,6-hexanediyl and the branched isomers thereof such as, 2-methylpentanediyl, 3-methylpentanediyl, 2,2-dimethylbutanediyl, 2,3-dimethylbutanediyl and the like; trihalomethyl defines
5 methyl containing three identical or different halo substituents for example trifluoromethyl; C_{2-6} alkenyl defines straight and branched chain hydrocarbon radicals containing one double bond and having from 2 to 6 carbon atoms such as, for example, ethenyl, 2-propenyl, 3-butenyl, 2-pentenyl, 3-pentenyl, 3-methyl-2-butenyl, and the like; and C_{3-10} cycloalkyl includes cyclic hydrocarbon groups having from 3 to 10
10 carbons, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctyl and the like.

The term “addition salt” comprises the salts which the compounds of formula (I) are able to form with organic or inorganic bases such as amines, alkali metal bases and
15 earth alkaline metal bases, or quaternary ammonium bases, or with organic or inorganic acids, such as mineral acids, sulfonic acids, carboxylic acids or phosphorus containing acids.

The term “addition salt” further comprises pharmaceutically acceptable salts, metal
20 complexes and solvates and the salts thereof, that the compounds of formula (I) are able to form.

The term “pharmaceutically acceptable salts” means pharmaceutically acceptable acid or base addition salts. The pharmaceutically acceptable acid or base addition salts as
25 mentioned hereinabove are meant to comprise the therapeutically active non-toxic acid and non-toxic base addition salt forms which the compounds of formula (I) are able to form. The compounds of formula (I) which have basic properties can be converted in their pharmaceutically acceptable acid addition salts by treating said base form with an appropriate acid. Appropriate acids comprise, for example, inorganic acids such as
30 hydrohalic acids, e.g. hydrochloric or hydrobromic acid; sulfuric; nitric; phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic (*i.e.* butanedioic acid), maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, cyclamic, salicylic, *p*-aminosalicylic, pamoic and the like acids.

35 The compounds of formula (I) which have acidic properties may be converted in their pharmaceutically acceptable base addition salts by treating said acid form with a suitable organic or inorganic base. Appropriate base salt forms comprise, for example,

the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, *N*-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

- 5 The terms acid or base addition salt also comprise the hydrates and the solvent addition forms which the compounds of formula (I) are able to form. Examples of such forms are e.g. hydrates, alcoholates and the like.

- 10 The term "metal complexes" means a complex formed between a compound of formula (I) and one or more organic or inorganic metal salt or salts. Examples of said organic or inorganic salts comprise the halogenides, nitrates, sulfates, phosphates, acetates, trifluoroacetates, trichloroacetates, propionates, tartrates, sulfonates, e.g. methylsulfonates, 4-methylphenylsulfonates, salicylates, benzoates and the like of the metals of the second main group of the periodical system, e.g. the magnesium or
15 calcium salts, of the third or fourth main group, e.g. aluminium, tin, lead as well as the first to the eighth transition groups of the periodical system such as, for example, chromium, manganese, iron, cobalt, nickel, copper, zinc and the like.

- The term stereochemically isomeric forms of compounds of formula (I), as used
20 hereinbefore, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds of formula (I) may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms which said compound may
25 possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. As is common in pharmacology one enantiomer can have a better pharmacological activity than the other. All stereochemically isomeric forms of the compounds of formula (I) both in pure form or in admixture with each other are intended to be embraced within the scope of the present invention.

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The *N*-oxide forms of the compounds of formula (I) are meant to comprise those compounds of formula (I) wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide, particularly those *N*-oxides wherein one or more of the piperidine-, piperazine or pyridazinyl-nitrogens are *N*-oxidized.

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Whenever used hereinafter, the term "compounds of formula (I)" is meant to include also the *N*-oxide forms, the pharmaceutically acceptable acid or base addition salts and

all stereoisomeric forms.

The compounds described in EP 371564 suppress the plasma elimination of retinoic acids. Unexpectedly, it has been found that the compounds of the present invention
5 show PARP inhibitory activity.

A first group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

- a) X is -N= or -CH= ;
- 10 b) R^1 is C_{1-6} alkyl;
- c) R^3 is hydrogen, C_{1-6} alkyl, a radical selected from (a-1), (a-2), (a-3) or (a-4) or a group of formula (b-1);
- d) R^6 is $\text{di(C}_{1-6}\text{alkyl)aminoC}_{1-6}\text{alkyl}$ or $\text{C}_{1-6}\text{alkyloxyC}_{1-6}\text{alkyl}$;
- e) R^7 is hydrogen;
- 15 f) R^8 is $\text{di(C}_{1-6}\text{alkyl)aminoC}_{1-6}\text{alkyl}$;
- g) t is 0 or 2;
- h) Z is a heterocyclic ring system selected from (c-1), (c-5), (c-6), (c-8), (c-10), (c-12) or (c-13);
- i) each R^{10} independently is hydrogen, C_{1-6} alkyl, hydroxy, $\text{C}_{1-6}\text{alkyloxyC}_{1-6}\text{alkyl}$,
20 $\text{C}_{1-6}\text{alkyloxyC}_{1-6}\text{alkylamino}$, morpholino, $\text{C}_{1-6}\text{alkylimidazolyl}$, or
pyridinyl $\text{C}_{1-6}\text{alkylamino}$;
- j) each R^{11} independently is hydrogen or hydroxy; and
- k) aryl is phenyl.

25 A second group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

- a) n is 0;
- b) X is CH;
- c) Q is -NH- , $\text{-CH}_2\text{-CH}_2\text{-}$ or $\text{-CHR}^5\text{-}$, wherein R^5 is hydrogen, hydroxy, or
30 aryl $\text{C}_{1-6}\text{alkyl}$;
- d) R^1 is C_{1-6} alkyl;
- e) R^2 is hydrogen;
- f) R^3 is hydrogen, hydroxy or a group of formula (b-1);
- g) t is 0;
- 35 h) Z is a heterocyclic ring system selected from (c-8) or (c-13);
- i) each R^{10} independently is hydrogen;
- j) aryl is phenyl.

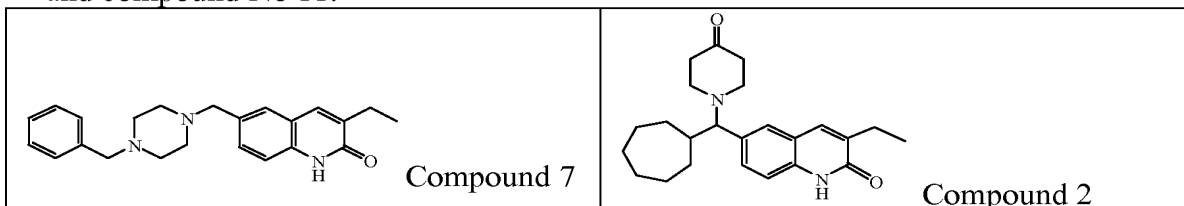
A third group of interesting compounds consists of those compounds of formula (I), the first group of interesting compounds or the second group of interesting compounds wherein Z is a heterocyclic ring system other than the heterocyclic ring system of formula (c-2) or (c-4).

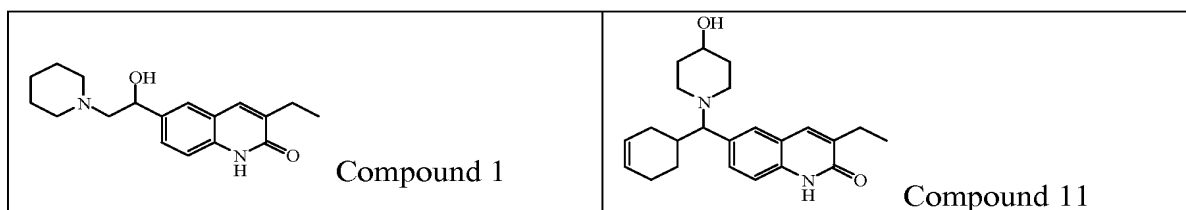
A group of preferred compounds consists of those compounds of formula (I) wherein X is $-N=$ or $-CH=$; R^1 is C_{1-6} alkyl; R^3 is hydrogen, C_{1-6} alkyl, a radical selected from (a-1), (a-2), (a-3) or (a-4) or a group of formula (b-1); R^6 is di(C_{1-6} alkyl)amino C_{1-6} alkyl or C_{1-6} alkyloxy C_{1-6} alkyl; R^7 is hydrogen; R^8 is di(C_{1-6} alkyl)amino C_{1-6} alkyl; t is 0 or 2; Z is a heterocyclic ring system selected from (c-1), (c-5), (c-6), (c-8), (c-10), (c-12) or (c-13); each R^{10} independently is hydrogen, C_{1-6} alkyl, hydroxy, C_{1-6} alkyloxy C_{1-6} alkyl, C_{1-6} alkyloxy C_{1-6} alkylamino, morpholino, C_{1-6} alkylimidazolyl, or pyridinyl C_{1-6} alkylamino; each R^{11} independently is hydrogen or hydroxy; and aryl is phenyl.

A further group of preferred compounds consists of those compounds of formula (I) wherein n is 0; X is CH; Q is $-NH-$, $-CH_2-CH_2-$ or $-CHR^5-$, wherein R^5 is hydrogen, hydroxy, or aryl C_{1-6} alkyl; R^1 is C_{1-6} alkyl; R^2 is hydrogen; R^3 is hydrogen, hydroxy or a group of formula (b-1); t is 0; Z is a heterocyclic ring system selected from (c-8) or (c-13); each R^{10} independently is hydrogen; and aryl is phenyl.

An even further group of preferred compounds consists of those compounds of formula (I), the group of preferred compounds or the further group of preferred compounds wherein Z is a heterocyclic ring system other than the heterocyclic ring system of formula (c-2) or (c-4).

The most preferred compounds are compound No 7, compound No 2, compound No 1 and compound No 11.

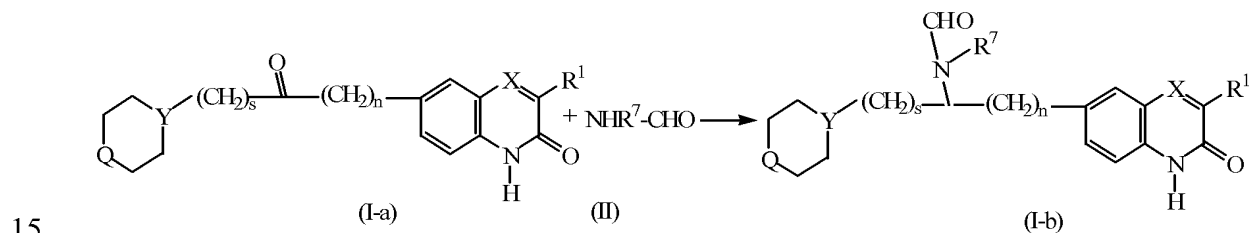




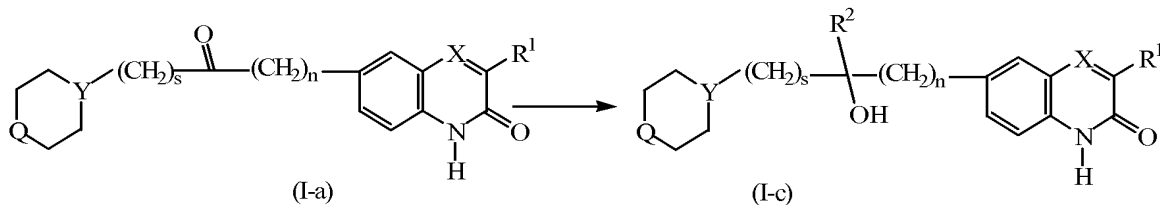
The compounds of formula (I) can be prepared according to the general methods described in EP 371564.

- 5 A number of such preparation methods will be described hereinafter in more detail. Other methods for obtaining final compounds of formula (I) are described in the examples.

- 10 Compounds of formula (I) wherein R^2 is hydrogen and R^3 is $-NR^7-CHO$ wherein R^7 is hydrogen or methyl, herein referred to as compounds of formula (I-b), can be prepared starting from compounds of formula (I), wherein R^2 taken together with R^3 forms $=O$, herein referred to as compounds of formula (I-a), in the presence of formamide or methylformamide, here indicated as intermediates of formula (II), and formic acid.

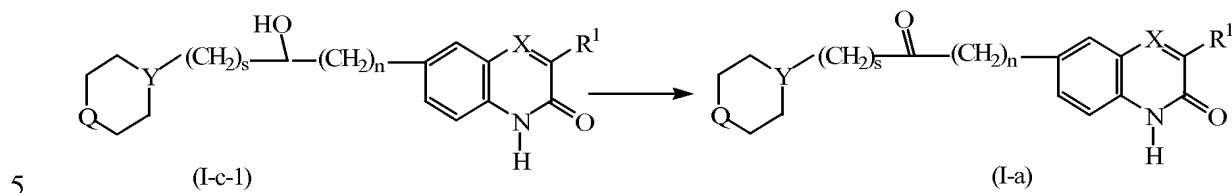


- 20 Compounds of formula (I), wherein R^3 is hydroxy, herein referred to as compounds of formula (I-c), can be prepared by converting the keton moiety of compounds of formula (I-a) into an hydroxy group, with an appropriate reductant, e.g., sodium borohydride in a suitable solvent, e.g. methanol and tetrahydrofuran.



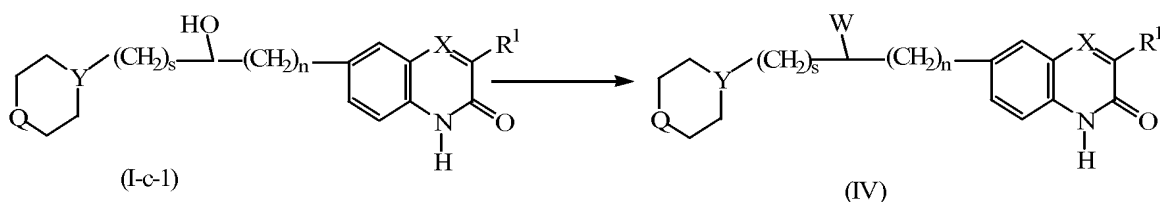
Compounds of formula (I-a) can be prepared by converting compounds of formula

(I-c), wherein R^2 is hydrogen, herein referred to as compounds of formula (I-c-1), in the presence of a suitable oxidant such as chromium trioxide and an acid such as sulfuric acid, in a suitable solvent such as 2-propanone.



Intermediates of formula (IV), wherein W is an appropriate leaving group such as, for example, chloro, bromo, methanesulfonyloxy or benzenesulfonyloxy can be prepared from compounds of formula (I-c-1) by treating said compounds with a suitable reagent e.g. methanesulfonyloxy chloride or benzenesulfonyloxy chloride, or a halogenating reagent such as e.g. $POCl_3$ or $SOCl_2$.

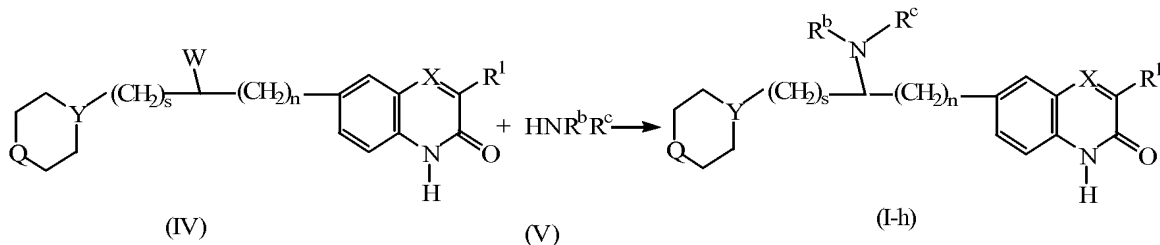
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Compounds of formula (I), defined as compounds of formula (I) wherein R^b is as defined in R^6 and R^c is as defined in R^7 , or R^b and R^c taken together with the nitrogen to which they are attached, form an appropriate heterocyclic ring system as defined in Z, herein referred to as compounds of formula (I-h), can be prepared by reacting an intermediate of formula (IV) with an intermediate of formula (V). The reaction can be performed in a reaction-inert solvent such as dimethylformamide or acetonitrile, and optionally in the presence of a suitable base such as, for example, sodium carbonate, potassium carbonate or triethylamine.

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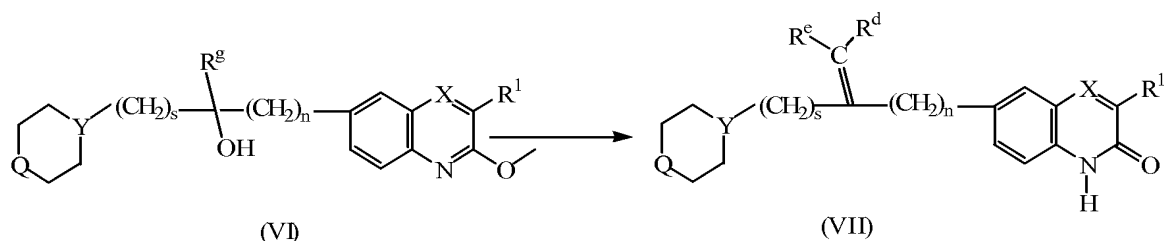


The compounds of formula (I) may also be converted into each other via art-known reactions or functional group transformations. A number of such transformations are already described hereinabove. Other examples are hydrolysis of carboxylic esters to the corresponding carboxylic acid or alcohol; hydrolysis of amides to the corresponding
5 carboxylic acids or amines; hydrolysis of nitriles to the corresponding amides; amino groups on imidazole or phenyl may be replaced by a hydrogen by art-known diazotation reactions and subsequent replacement of the diazo-group by hydrogen; alcohols may be converted into esters and ethers; primary amines may be converted into secondary or tertiary amines; double bonds may be hydrogenated to the
10 corresponding single bond; an iodo radical on a phenyl group may be converted in to an ester group by carbon monoxide insertion in the presence of a suitable palladium catalyst.

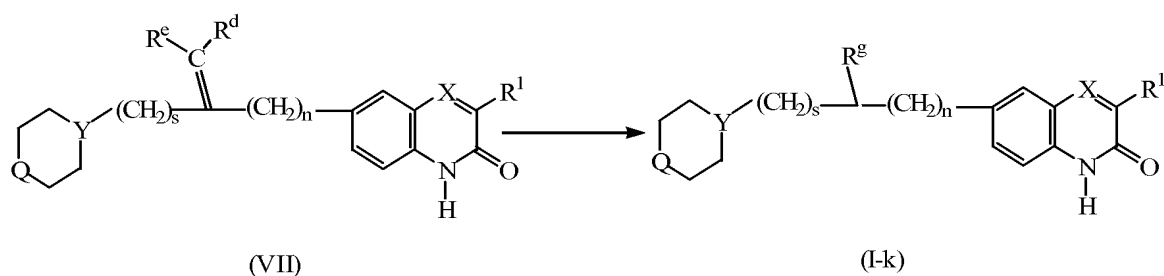
Hence, compounds of formula (I), (I-a), (I-b), (I-c), (I-c-1), (I-h), (I-i), (I-j) and (I-k)
15 can optionally be the subject of one or more of the following conversions in any desired order:

- (i) converting a compound of formula (I) into a different compound of formula (I);
- (ii) converting a compound of formula (I) into the corresponding acceptable salt or N-oxide thereof;
- 20 (iii) converting a pharmaceutically acceptable salt or N-oxide of a compound of formula (I) into the parent compound of formula (I);
- (iv) preparing a stereochemical isomeric form of a compound of formula (I) or a pharmaceutically acceptable salt or N-oxide thereof.

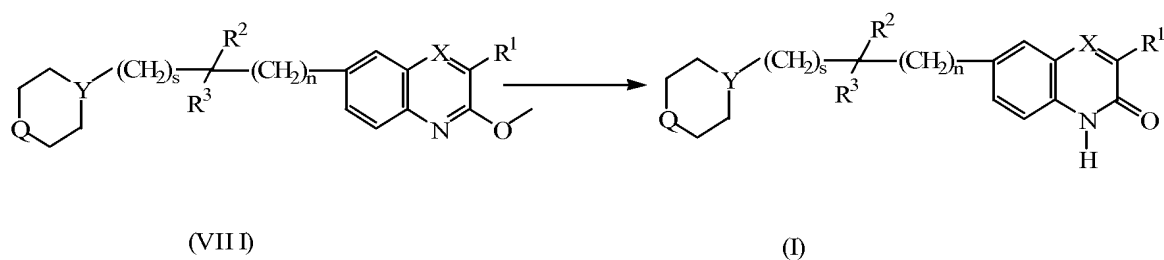
25 Intermediates of formula (VII), wherein R^d and R^e are appropriate radicals or taken together with the carbon to which they are attached, form an appropriate heterocyclic ring system as defined in Z, can be prepared by hydrolysing intermediates of formula (VI), wherein R^3 is a group of formula (b-1) or a radical of formula (a-1) wherein s is other than 0, herein referred to as R^g , according to art-known methods, such as stirring
30 the intermediate (VI) in an aqueous acid solution in the presence of a reaction inert solvent, e.g. tetrahydrofuran. An appropriate acid is for instance hydrochloric acid.



Compounds of formula (I) wherein R^2 is hydrogen and R^g is as defined above, herein referred to as compounds of formula (I-k), can be prepared starting from intermediates of formula (VII), by a selective hydrogenation of said intermediate with an appropriate reducing agent such as, for example with a noble catalyst, such as platinum-on-charcoal, palladium-on-charcoal and the like and an appropriate reductant such as hydrogen in a suitable solvent such as methanol.



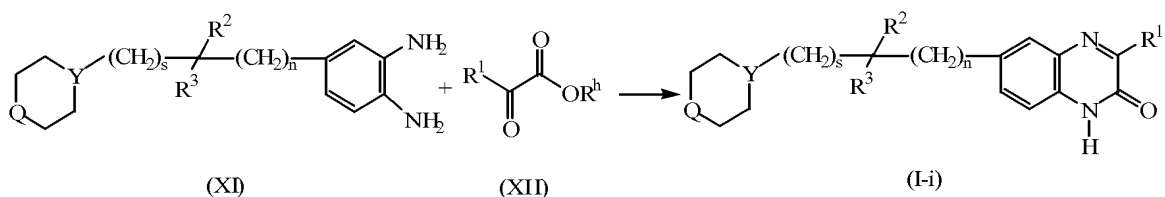
Compounds of formula (I) can be prepared by hydrolysing intermediates of formula (VIII), according to art-known methods, by submitting the intermediates of formula (VIII) to appropriate reagents, such as, tinchloride, acetic acid and hydrochloric acid, in the presence of a reaction inert solvent, e.g. tetrahydrofuran.



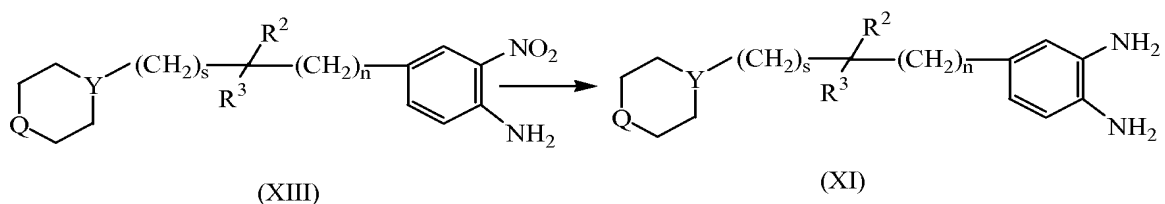
Compounds of formula (I) can be prepared starting from N-oxides of formula (IX) by converting the intermediates of formula (IX) in the presence of a suitable reagent such as sodium carbonate or acetic anhydride and when appropriate in a solvent such as dichloromethane.

(X) (I-j)

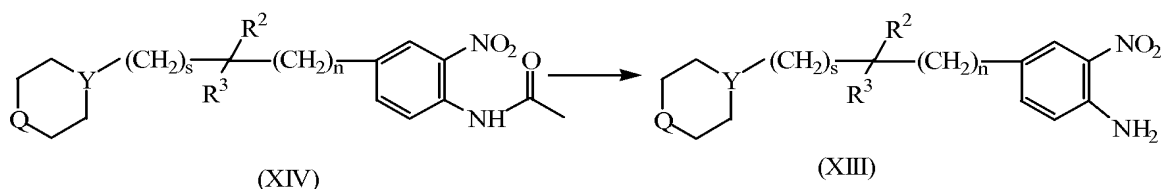
15 The compounds of formula (I), wherein X is N, herein referred to as compounds of
formula (I-i) may be obtained by condensing an appropriate ortho-benzenediamine of
formula (XI) with an ester of formula (XII) wherein R^h is C₁₋₆alkyl. The condensation
of the substituted ortho-diamine of formula (XI) and the ester of formula (XII) can be
20 carried out in the presence of a carboxylic acid, e.g. acetic acid and the like, a mineral
acid such as, for example hydrochloric acid, sulfuric acid, or a sulfonic acid such as, for
example, methanesulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfonic acid
and the like. Somewhat elevated temperatures may be appropriate to enhance the rate
of the reaction and in some cases the reaction may even be carried out at the reflux
25 temperature of the reaction mixture. The water which is liberated during the
condensation may be removed from the mixture by azeotropic distillation, distillation
and the like methods.



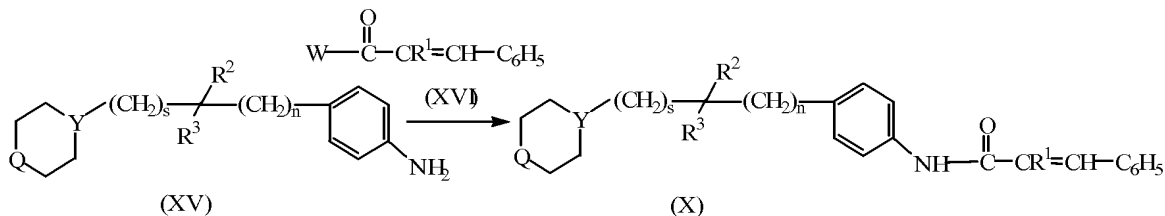
- Intermediates of formula (XI) can be prepared by a nitro to amine reduction reaction starting with an intermediate of formula (XIII) in the presence of a metal catalyst such as Raney Nickel and an appropriate reductant such as hydrogen in a suitable solvent such as methanol.



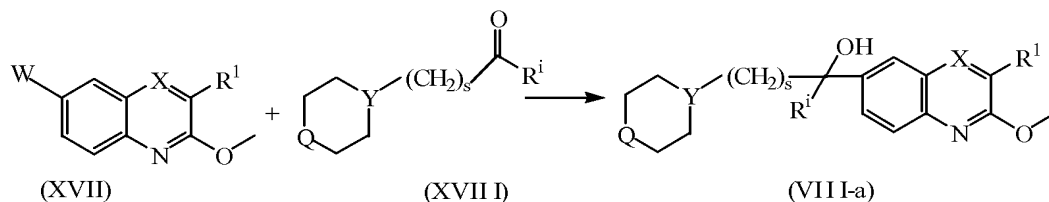
- Intermediates of formula (XIII) can be prepared by hydrolysing intermediates of formula (XIV), according to art-known methods, such as stirring the intermediate (XIV) in an aqueous acid solution in the presence of a reaction inert solvent, e.g. tetrahydrofuran,. An appropriate acid is for instance hydrochloric acid.



- Intermediates of formula (X) can conveniently be prepared by reacting an aniline of formula (XV) with a halide of formula (XVI) in the presence of a base such as pyridine in a suitable solvent such as dichloromethane.



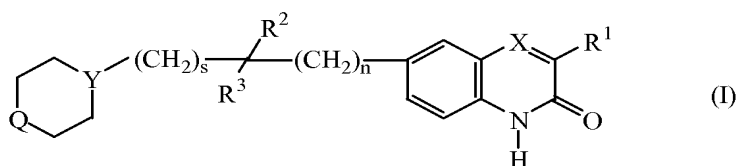
Intermediates of formula (VIII) wherein n is 0, R^2 is hydrogen or hydroxy and when R^2 is hydrogen then R^3 is hydroxy herein referred to as intermediates of formula (VIII-a) can be prepared by treating an intermediate of formula (XVII), wherein W is halo, with an organolithium reagent such as, e.g. *n*-butyllithium in a reaction inert solvent, e.g. tetrahydrofuran, and subsequently reacting said intermediate with an intermediate of formula (XVIII) wherein R^1 is hydrogen or a radical as defined in R^3 .



The present invention also relates to a compound of formula (I) as defined above for use as a medicine.

The compounds of the present invention have PARP inhibiting properties as can be seen from the experimental part hereinunder.

The present invention also contemplates the use of compounds in the preparation of a medicament for the treatment of any of the diseases and disorders in an animal described herein, wherein said compounds is a compound of formula (I)



the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

n is 0 or 1;

s is 0 or 1;

X is $-N=$ or $-CR^4=$, wherein R^4 is hydrogen or taken together with R^1 may form a bivalent radical of formula $-CH=CH-CH=CH-$;

Y is $-N<$ or $-CH<$;

Q is $-\text{NH}-$, $-\text{O}-$, $-\text{C}(\text{O})-$, $-\text{CH}_2\text{CH}_2-$ or $-\text{CHR}^5-$,
 wherein R^5 is hydrogen, hydroxy, C_{1-6} alkyl, aryl C_{1-6} alkyl, C_{1-6} alkyloxycarbonyl,
 C_{1-6} alkyloxy C_{1-6} alkylamino or haloindazolyl;

5 R^1 is C_{1-6} alkyl or thienyl;

R^2 is hydrogen or taken together with R^3 may form $=\text{O}$;

R^3 is hydrogen, C_{1-6} alkyl or a radical selected from

- 10 $-\text{NR}^6\text{R}^7$ (a-1),
 $-\text{O}-\text{H}$ (a-2),
 $-\text{O}-\text{R}^8$ (a-3),
 $-\text{S}-\text{R}^9$ (a-4), or
 $-\text{C}\equiv\text{N}$ (a-5),

15 wherein

R^6 is $-\text{CHO}$, C_{1-6} alkyl, hydroxy C_{1-6} alkyl, C_{1-6} alkylcarbonyl,
 di(C_{1-6} alkyl)amino C_{1-6} alkyl, C_{1-6} alkylcarbonylamino C_{1-6} alkyl,
 piperidiny C_{1-6} alkyl, piperidinyl C_{1-6} alkylaminocarbonyl, C_{1-6} alkyloxy,
 C_{1-6} alkyloxy C_{1-6} alkyl, thienyl C_{1-6} alkyl, pyrrolyl C_{1-6} alkyl,
 20 aryl C_{1-6} alkylpiperidinyl, arylcarbonyl C_{1-6} alkyl, arylcarbonylpiperidinyl C_{1-6} alkyl,
 haloindozolylpiperidinyl C_{1-6} alkyl, or aryl C_{1-6} alkyl(C_{1-6} alkyl)amino C_{1-6} alkyl; and
 R^7 is hydrogen or C_{1-6} alkyl;
 R^8 is C_{1-6} alkyl, C_{1-6} alkylcarbonyl or di(C_{1-6} alkyl)amino C_{1-6} alkyl; and
 R^9 is di(C_{1-6} alkyl)amino C_{1-6} alkyl;

25 or R^3 is a group of formula

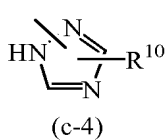
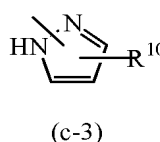
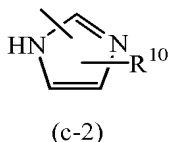
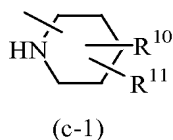


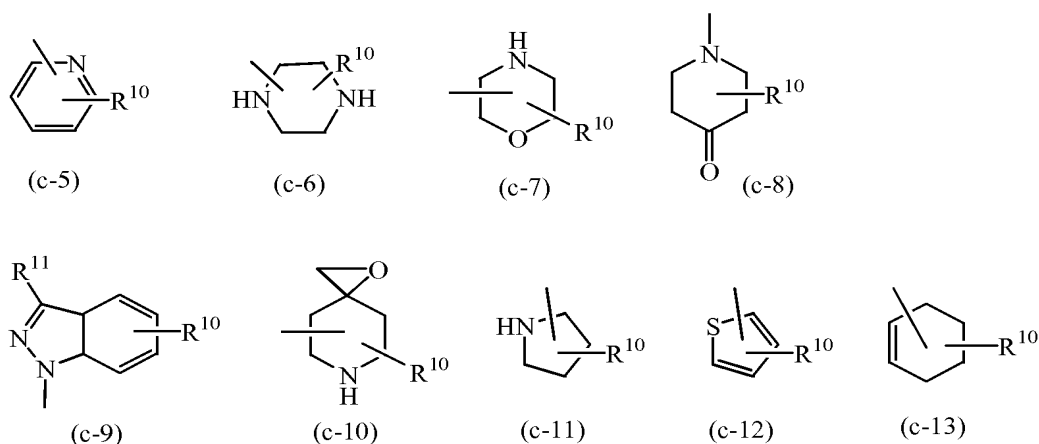
wherein

t is 0, 1 or 2;

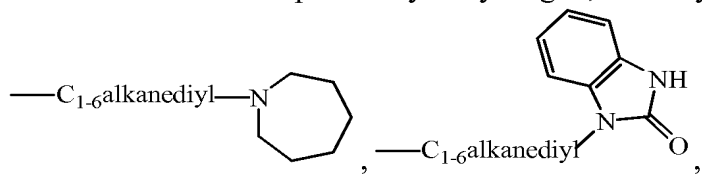
Z is a heterocyclic ring system selected from

30





5 wherein each R^{10} independently is hydrogen, C_{1-6} alkyl, aminocarbonyl, hydroxy,



C_{1-6} alkyloxy C_{1-6} alkyl, C_{1-6} alkyloxy C_{1-6} alkylamino, di(phenyl C_{2-6} alkenyl),
 piperidiny1 C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{3-10} cycloalkyl C_{1-6} alkyl,
 aryloxy(hydroxy) C_{1-6} alkyl, haloindazolyl, aryl C_{1-6} alkyl, aryl C_{2-6} alkenyl,
 10 morpholino, C_{1-6} alkylimidazolyl, or pyridiny1 C_{1-6} alkylamino;
 each R^{11} independently is hydrogen, hydroxy, piperidiny1 or aryl;

aryl is phenyl or phenyl substituted with halo, C_{1-6} alkyl or C_{1-6} alkyloxy.

15 In view of their PARP binding properties the compounds of the present invention
 may be used as reference compounds or tracer compounds in which case one of the
 atoms of the molecule may be replaced with, for instance, a radioactive isotope.

To prepare the pharmaceutical compositions of this invention, an effective amount of a
 20 particular compound, in base or acid addition salt form, as the active ingredient is
 combined in intimate admixture with a pharmaceutically acceptable carrier, which
 carrier may take a wide variety of forms depending on the form of preparation desired
 for administration. These pharmaceutical compositions are desirably in unitary dosage
 form suitable, preferably, for administration orally, rectally, percutaneously, or by
 25 parenteral injection. For example, in preparing the compositions in oral dosage form,
 any of the usual pharmaceutical media may be employed, such as, for example, water,
 glycols, oils, alcohols and the like in the case of oral liquid preparations such as

suspensions, syrups, elixirs and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid

5 pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, to aid solubility for example, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be

10 prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not cause a significant deleterious effect to the skin. Said additives may

15 facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on, as an ointment. It is especially advantageous to formulate the aforementioned pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used in the

20 specification and claims herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, injectable solutions or

25 suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

The compounds of the present invention can treat or prevent tissue damage resulting from cell damage or death due to necrosis or apoptosis; can ameliorate neural or

30 cardiovascular tissue damage, including that following focal ischemia, myocardial infarction, and reperfusion injury; can treat various diseases and conditions caused or exacerbated by PARP activity; can extend or increase the lifespan or proliferative capacity of cells; can alter the gene expression of senescent cells; can radiosensitize and/or chemosensitize cells. Generally, inhibition of PARP activity spares the cells

35 from energy loss, preventing, in the case of neural cells, irreversible depolarization of the neurons, and thus, provides neuroprotection.

For the foregoing reasons, the present invention further relates to a method of administering a therapeutically effective amount of the above-identified compounds in an amount sufficient to inhibit PARP activity, to treat or prevent tissue damage resulting from cell damage or death due to necrosis or apoptosis, to effect a neuronal activity not mediated by NMDA toxicity, to effect a neuronal activity mediated by NMDA toxicity, to treat neural tissue damage resulting from ischemia and reperfusion injury, neurological disorders and neurodegenerative diseases; to prevent or treat vascular stroke; to treat or prevent cardiovascular disorders; to treat other conditions and/or disorders such as age- related muscular degeneration, AIDS and other immune senescence diseases, inflammation, gout, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, inflammatory bowel disorders (such as colitis and Crohn's disease), muscular dystrophy, osteoarthritis, osteoporosis, chronic and/or acute pain (such as neuropathic pain), renal failure, retinal ischemia, septic shock (such as endotoxic shock), and skin aging, to extend the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; chemosensitize and/or radiosensitize (hypoxic) tumor cells. The present invention also relates to treating diseases and conditions in an animal which comprises administering to said animal a therapeutically effective amount of the above-identified compounds.

In particular, the present invention relates to a method of treating, preventing or inhibiting a neurological disorder in an animal, which comprises administering to said animal a therapeutically effective amount of the above-identified compounds. The neurological disorder is selected from the group consisting of peripheral neuropathy caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke associated with brain damage, focal ischemia, global ischemia, reperfusion injury, demyelinating disease and neurological disorder relating to neurodegeneration.

The present invention also contemplates the use of compounds of formula (I) for inhibiting PARP activity, for treating, preventing or inhibiting tissue damage resulting from cell damage or death due to necrosis or apoptosis, for treating, preventing or inhibiting a neurological disorder in an animal.

The term "preventing neurodegeneration" includes the ability to prevent neurodegeneration in patients newly diagnosed as having a neurodegenerative disease, or at risk of developing a new degenerative disease and for preventing further

neurodegeneration in patients who are already suffering from or have symptoms of a neurodegenerative disease.

5 The term "treatment" as used herein covers any treatment of a disease and/or condition in an animal, particularly a human, and includes: (i) preventing a disease and/or condition from occurring in a subject which may be predisposed to the disease and/or condition but has not yet been diagnosed as having it; (ii) inhibiting the disease and/or condition, i.e., arresting its development; (iii) relieving the disease and/or condition, i.e., causing regression of the disease and/or condition.

10

The term "radiosensitizer", as used herein, is defined as a molecule, preferably a low molecular weight molecule, administered to animals in therapeutically effective amounts to increase the sensitivity of the cells to ionizing radiation and/or to promote the treatment of diseases which are treatable with ionizing radiation. Diseases which
15 are treatable with ionizing radiation include neoplastic diseases, benign and malignant tumors, and cancerous cells. Ionizing radiation treatment of other diseases not listed herein are also contemplated by the present invention.

The term "chemosensitizer", as used herein, is defined as a molecule, preferably a low
20 molecular weight molecule, administered to animals in therapeutically effective amounts to increase the sensitivity of cells to chemotherapy and/or promote the treatment of diseases which are treatable with chemotherapeutics. Diseases which are treatable with chemotherapy include neoplastic diseases, benign and malignant tumors and cancerous cells. Chemotherapy treatment of other diseases not listed herein are also
25 contemplated by the present invention.

The compounds, compositions and methods of the present invention are particularly useful for treating or preventing tissue damage resulting from cell death or damage due to necrosis or apoptosis.

30 The compounds of the present invention can be "anti-cancer agents", which term also encompasses "anti-tumor cell growth agents" and "anti-neoplastic agents". For example, the methods of the invention are useful for treating cancers and chemosensitizing and/or radiosensitizing tumor cells in cancers such as ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia,
35 cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma

gallbladder cancer, hairy cell leukemia, head & neck cancer, Hodgkin's Lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and/or non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non- Hodgkin's Lymphoma,

5 osteosarcoma, ovarian cancer, ovary (germ cell) cancer, prostate cancer, pancreatic cancer, penile cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva and Wilm's tumor.

10 Hence the compounds of the present invention can be used as "radiosensitizer" and/or "chemosensitizer".

Radiosensitizers are known to increase the sensitivity of cancerous cells to the toxic effects of ionizing radiation. Several mechanisms for the mode of action of
15 radiosensitizers have been suggested in the literature including: hypoxic cell radiosensitizers (e.g., 2- nitroimidazole compounds, and benzotriazine dioxide compounds) mimicking oxygen or alternatively behave like bioreductive agents under hypoxia; non-hypoxic cell radiosensitizers (e.g., halogenated pyrimidines) can be analogs of DNA bases and preferentially incorporate into the DNA of cancer cells and
20 thereby promote the radiation-induced breaking of DNA molecules and/or prevent the normal DNA repair mechanisms; and various other potential mechanisms of action have been hypothesized for radiosensitizers in the treatment of disease.

Many cancer treatment protocols currently employ radiosensitizers in conjunction with radiation of x-rays. Examples of x-ray activated radiosensitizers include, but are not
25 limited to, the following: metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, EO9, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5- iododeoxyuridine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FudR), hydroxyurea, cisplatin, and therapeutically effective analogs and derivatives of the same.

30 Photodynamic therapy (PDT) of cancers employs visible light as the radiation activator of the sensitizing agent. Examples of photodynamic radiosensitizers include the following, but are not limited to: hematoporphyrin derivatives, Photofrin, benzoporphyrin derivatives, tin etioporphyrin, pheoborbide-a, bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanine, and therapeutically effective
35 analogs and derivatives of the same.

Radiosensitizers may be administered in conjunction with a therapeutically effective amount of one or more other compounds, including but not limited to: compounds

which promote the incorporation of radiosensitizers to the target cells; compounds which control the flow of therapeutics, nutrients, and/or oxygen to the target cells; chemotherapeutic agents which act on the tumor with or without additional radiation; or other therapeutically effective compounds for treating cancer or other disease.

- 5 Examples of additional therapeutic agents that may be used in conjunction with radiosensitizers include, but are not limited to: 5-fluorouracil, leucovorin, 5' -amino-5'deoxythymidine, oxygen, carbogen, red cell transfusions, perfluorocarbons (e.g., Fluosol 10 DA), 2,3-DPG, BW12C, calcium channel blockers, pentoxifylline, antiangiogenesis compounds, hydralazine, and LBSO. Examples of chemotherapeutic
- 10 agents that may be used in conjunction with radiosensitizers include, but are not limited to: adriamycin, camptothecin, carboplatin, cisplatin, daunorubicin, docetaxel, doxorubicin, interferon (alpha, beta, gamma), interleukin 2, irinotecan, paclitaxel, topotecan, and therapeutically effective analogs and derivatives of the same.
- 15 Chemosensitizers may be administered in conjunction with a therapeutically effective amount of one or more other compounds, including but not limited to : compounds which promote the incorporation of chemosensitizers to the target cells; compounds which control the flow of therapeutics, nutrients, and/or oxygen to the target cells; chemotherapeutic agents which act on the tumor or other therapeutically effective
- 20 compounds for treating cancer or other disease. Examples of additional therapeutic agents that may be used in conjunction with chemosensitizers include, but are not limited to : methylating agents, topoisomerase I inhibitors and other chemotherapeutic agents such as cisplatin and bleomycin.
- 25 The compounds of formula (I) can also be used to detect or identify the PARP, and more in particular the PARP-1 receptor. For that purpose the compounds of formula (I) can be labeled. Said label can be selected from the group consisting of a radioisotope, spin label, antigen label, enzyme label fluorescent group or a chemiluminiscent group.
- 30 Those skilled in the art could easily determine the effective amount from the test results presented hereinafter. In general it is contemplated that an effective amount would be from 0.01 mg/kg to 100 mg/kg body weight, and in particular from 0.05 mg/kg to 10 mg/kg body weight. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses
- 35 may be formulated as unit dosage forms, for example, containing 0.5 to 500 mg, and in particular 1 mg to 200 mg of active ingredient per unit dosage form.

The following examples illustrate the present invention.

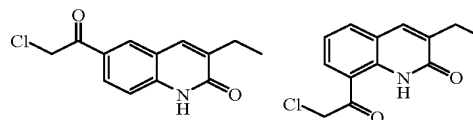
Experimental part

Hereinafter, "BuLi" is defined as butyl-lithium, "DCM" is defined as dichloromethane, "DIPE" is defined as diisopropyl ether, "DMF" is defined as *N,N*-dimethylformamide, "DMSO" is defined as dimethylsulfoxide, "EtOAc" is defined as ethyl acetate, "EtOH" is defined as ethanol, "MEK" is defined as methyl ethyl keton, "MeOH" is defined as methanol and "THF" is defined as tetrahydrofuran.

10 A. Preparation of the intermediate compounds

Example A1

a) Preparation of intermediate 1 and 2

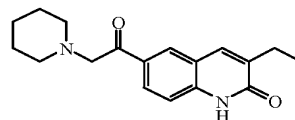


intermediate 1

intermediate 2

Aluminium chloride (0.6928 mol) was added portionwise to a solution of chloro- acetyl chloride (0.5196 mol) in DCM (50.2ml) while the temperature was kept below 30°C. 3-ethyl- 2(1*H*)-quinolinone (0.1732 mol) was added while the temperature was kept below 30°C. The mixture was stirred and refluxed for 15 hours, cooled and poured out into ice water. The precipitate was filtered off, washed with water and taken up in DCM. The organic solution was stirred and filtered. The precipitate was dried, yielding 33.5g of intermediate 1. The filtrate was extracted. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated till dryness, yielding 20.46g of intermediate 2.

b) Preparation of intermediate 3

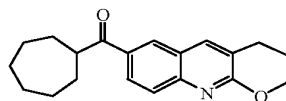


Piperidine (0.24 mol) was added dropwise at room temperature to a solution of intermediate 1 and intermediate 2 in DMF (300ml). The mixture was stirred for 5 min, poured out into water and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated till dryness. The residue (39.14g) was purified by column chromatography over silica gel (20-45 μ m) (eluent: DCM/MeOH/NH₄OH 96/4/0.2). The pure fractions were collected and the solvent was evaporated. Part (3.7g) of the residue (13.8g) was crystallized from 2-propanone. The

precipitate was filtered off, washed with diethyl ether and dried, yielding 3g of intermediate 3, melting point 190°C.

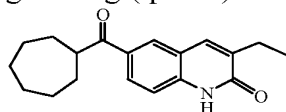
Example A2

a) Preparation of intermediate 4



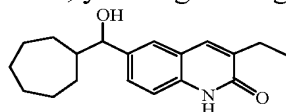
- 5 nBuLi 1.6M in hexane (0.0764 mol) was added dropwise at -60°C under N_2 flow to a mixture of 6-bromo-3-ethyl-2-methoxy-quinoline (0.0694 mol) in THF (185ml). The mixture was stirred at -60°C for 1 hour and then added dropwise at -60°C to a mixture of *N*-methoxy-*N*-methyl-cycloheptanecarboxamide (0.0694 mol) in diethyl ether (100ml). The mixture was stirred at -60°C for 1 hour, then brought to 0°C , poured out
- 10 into a saturated NH_4Cl solution and extracted with EtOAc. The organic layer was separated, dried (MgSO_4), filtered and the solvent was evaporated. The product was used without further purification, yielding 21.61g (quant.) of intermediate 4.

b) Preparation of intermediate 5



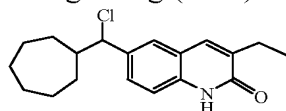
- A mixture of intermediate 4 (0.0694 mol) in hydrochloric acid 3N (317ml) and THF (159ml) was stirred and refluxed overnight. The mixture was poured out on ice,
- 15 basified with a concentrated NH_4OH solution and extracted with EtOAc. The organic layer was separated, dried (MgSO_4), filtered and the solvent was evaporated. The product was used without further purification, yielding 17.59g (85%) of intermediate 5.

c) Preparation of intermediate 6



- Sodium hydroborate (0.0296 mol) was added portionwise at 0°C under N_2 flow to a mixture of intermediate 5 (0.0591 mol) in MeOH (176ml). The mixture was poured out
- 20 on ice and extracted with DCM. The precipitate was filtered off and dried. The product was used without further purification, yielding 6.38g (36%) of intermediate 6.

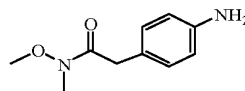
d) Preparation of intermediate 7



- Intermediate 6 (0.0213 mol) was added portionwise at 0°C to thionyl chloride (32ml). The mixture was stirred at room temperature overnight. The solvent was evaporated till dryness. The product was used without further purification, yielding 6.77g (quant.) of
- 25 intermediate 7.

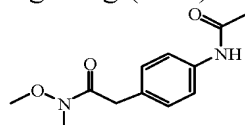
Example A3

a) Preparation of intermediate 8



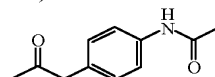
- A mixture of *N*-methoxy-*N*-methyl-4-nitro- benzeneacetamide (0.534 mol) in MeOH (1200ml) was hydrogenated at room temperature under a 3 bar pressure for 1 hour with Raney nickel (60g) as a catalyst. After uptake of H₂ (3 equiv), the catalyst was filtered off and the filtrate was evaporated, yielding 102g (98%) of intermediate 8.

b) Preparation of intermediate 9



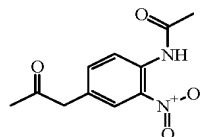
- Acetyl anhydride (1.36 mol) was added dropwise at room temperature to a mixture of intermediate 8 (0.525 mol) in DCM (100ml). The mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated.
- The residue (151.6g) was purified by column chromatography over silica gel (20-45 μm) (eluent: DCM/MeOH/NH₄OH 95/5/0.1). The pure fractions were collected and the solvent was evaporated, yielding 32g (26%) of intermediate 9.

c) Preparation of intermediate 10



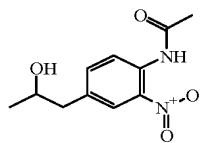
- Methyl- lithium 1.6M (74ml) was added at 0°C under N₂ flow to a mixture of intermediate 9 (0.059 mol) in THF (210ml). The mixture was stirred at 0°C for 90 min, poured out into water and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (20-45 μm) (eluent: DCM/MeOH/NH₄OH 96/4/0.1). The desired fractions were collected and the solvent was evaporated, yielding 7g (33%) of intermediate 10.

d) Preparation of intermediate 11



- Nitric acid fuming (5.6ml) was added dropwise at a temperature below 30°C to a mixture of intermediate 10 (0.037 mol) in acetyl anhydride (100ml). The mixture was stirred at a temperature below 30°C for 1 hour, poured out into ice water, basified with a concentrated NH₄OH solution and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15-35 μm) (eluent: DCM/MeOH 99/1). The desired fractions were collected and the solvent was evaporated, yielding 4g of intermediate 11.

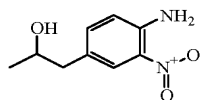
e) Preparation of intermediate 12



Sodium hydroborate (0.0187 mol) was added portionwise at 5°C to a mixture of intermediate 11 (0.017 mol) in MeOH (50ml). The mixture was stirred at 5°C, hydrolyzed with water and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated, yielding 4.2g (quant.) of

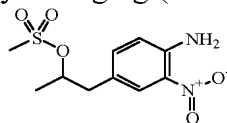
5 intermediate 12.

f) Preparation of intermediate 13



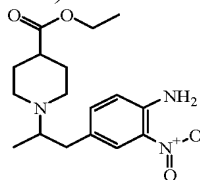
A mixture of intermediate 12 (0.0176 mol) in sodium hydroxide 2N (65ml), THF (25ml) and EtOH (25ml) was stirred at room temperature for 15 hours, poured out into water and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated, yielding 3g (86%) of intermediate 13.

g) Preparation of intermediate 14



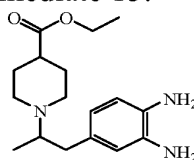
10 Triethylamine (0.03 mol) was added at room temperature to a mixture of intermediate 13 (0.015 mol) in DCM (40ml). Methanesulfonyl chloride (0.015 mol) was added at 0°C under N₂ flow. The mixture was stirred at 0°C for 1 hour and at room temperature for 3 hours. The solvent was evaporated at room temperature. The product was used without further purification, yielding (quant.) intermediate 14.

h) Preparation of intermediate 15



15 A mixture of intermediate 14 (0.015 mol), ethyl 4-piperidinecarboxylate (0.045 mol) and potassium carbonate (0.045 mol) in acetonitrile (100ml) was stirred and refluxed for 15 hours, poured out into water and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15-35 μm) (eluent: DCM/MeOH/NH₄OH 97/3/0.1). The pure fractions were collected and the solvent was evaporated, yielding 0.7g (14%) of intermediate 15.

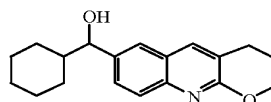
i) Preparation of intermediate 16



A mixture of intermediate 15 (0.002 mol) in MeOH (50ml) was hydrogenated at room temperature under a 3 bar pressure for 1 hour with Raney nickel (0.7g) as a catalyst. After uptake of H₂ (3 equiv), the catalyst was filtered through celite, washed with MeOH and a small amount of DCM and the filtrate was evaporated, yielding 0.65g (quant.) of intermediate 16.

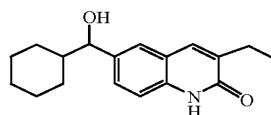
Example A4

Preparation of intermediate 17



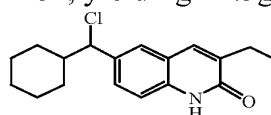
nBuLi 1.6 M in hexane (0.148 mol) was added dropwise at -60°C under N₂ flow to a mixture of 6-bromo-3-ethyl-2-methoxyquinoline (0.114 mol) in THF (500ml) and the mixture was stirred at -60°C for 1h. Cyclohexanecarboxaldehyde (0.137 mol) in THF (100ml) was added dropwise at -60°C, stirred at -60°C for 2h and then stirred further at -40°C for 1h. The mixture was poured into saturated NH₄Cl and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The product was used without further purification, yielding 34.13g (quant.) of intermediate 17.

b) Preparation of intermediate 18



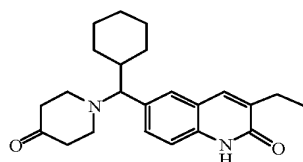
A mixture of intermediate 17 (0.114 mol) in hydrochloric acid 3N (250ml) and THF (250ml) was stirred and refluxed for 24 hours. The mixture was cooled and DCM (200ml) was added. The precipitate was filtered off, washed with water and dried. The product was used without further purification, yielding 12.5g (39%) of intermediate 18.

c) Preparation of intermediate 19



Intermediate 18 (0.035 mol) was added portionwise at 0°C to thionyl chloride (56.23ml). The mixture was stirred at room temperature for 3 hours and the solvent was evaporated. The residue was crystallized from diethyl ether. The precipitate was filtered off, washed several times with diethyl ether and recrystallized from water and DCM. The mixture was stirred for 15 hours. The precipitate was filtered off and dried, yielding 7.9g (75%) of intermediate 19.

d) Preparation of intermediate 20

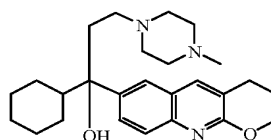


A solution of 4,4-piperidinediol, hydrochloride (0.0651 mol) and potassium carbonate (0.217 mol) in DMF (250ml) was stirred at room temperature under N₂ flow for 10 min. A solution of intermediate 19 (0.0434 mol) in DMF (50ml) was added slowly, and the mixture was stirred at room temperature for 1 hour and then at 70°C for one hour.

- 5 The mixture was cooled to room temperature and poured into water (1500ml). The precipitate was filtered off, washed several times with cold water and dried. The product was used in the next reaction step without further purification. A part (3g) of the residue (13.8g) was purified by column chromatography over silica gel (15-40 μ m) (eluent: DCM/MeOH/NH₄OH 99/1/0.1). The desired fractions were collected and the
- 10 solvent was evaporated. The residue (2.9g) was crystallized from MEK/DIPE, filtered off and dried, yielding 2.85g of intermediate 20.

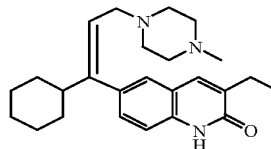
Example A5

a). Preparation of intermediate 21



- nBuLi 1.6M in hexane (0.0516 mol) was added dropwise at -60°C under N₂ flow to a
- 15 mixture of 6-bromo-3-ethyl-2-methoxy- quinoline (0.043 mol) in THF (115ml). The mixture was stirred at -60°C for 1 hour. A mixture of 1-cyclohexyl-3-(4-methyl-1-piperazinyl)- 1-propanone, (0.043 mol) in THF (103ml) was added dropwise at -60°C. The mixture was stirred at -60°C for 1 hour, brought to 0°C, poured out into a saturated
- 20 NH₄Cl solution and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (20-45 μ m) (eluent: DCM/MeOH/NH₄OH 97/3/0.2 and 90/10/0.2). The pure fractions were collected and the solvent was evaporated, yielding 3.7g (20%) of intermediate 21.

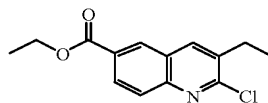
b). Preparation of intermediate 22



- A mixture of intermediate 21 (0.00841 mol), tin(II) chloride (0.0336 mol) and
- 25 hydrochloric acid 12N (0.121 mol) in acetic acid (36ml) was stirred at 80°C for 16 hours. The mixture was poured out on ice, basified with a concentrated NH₄OH solution and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The product was used without further purification, yielding 2.45g (74%) of intermediate 22.

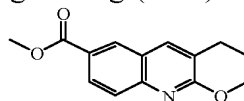
Example A6

a) Preparation of intermediate 23



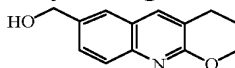
Phosphoryl chloride (110.9ml) was added dropwise at 5°C to DMF (81.5ml). The mixture was stirred until complete dissolution. 4-[(1-oxobutyl)amino]- benzoic acid, ethyl ester (0.34 mol) was added. The mixture was stirred at 100°C for 15 hours, then cooled to room temperature and poured out into ice water. The precipitate was filtered off, washed with water and dried, yielding 42.35g (47%) of intermediate 23.

b) Preparation of intermediate 24



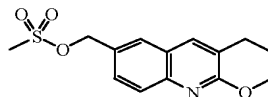
A mixture of intermediate 23 (0.1606 mol) in sodium methanolate 30% solution in MeOH (152.8ml) and MeOH (400ml) was stirred and refluxed for 15 hours, then cooled and poured out into ice water. The precipitate was filtered off, washed with water and taken up in DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated till dryness, yielding 31.64g (85%) of intermediate 24.

c) Preparation of intermediate 25



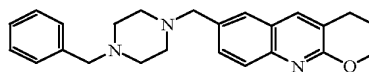
Lithium aluminum tetrahydride (0.1288 mol) was added portionwise at 0°C under N₂ flow to a solution of intermediate 24 (0.1288 mol) in THF (263ml). The mixture was stirred for 30 min, poured out into ice water and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated till dryness, yielding 27.4g (98%) of intermediate 25.

d) Preparation of intermediate 26



Methanesulfonyl chloride (0.104 mol) was added dropwise at 0°C under N₂ flow to a mixture of intermediate 25 (0.069 mol) and triethylamine (0.207 mol) in DCM (120ml). The mixture was stirred at 0°C for 4 hours. The solvent was evaporated till dryness (without heating). The product was used without further purification, yielding 20.4g of intermediate 26.

e) Preparation of intermediate 27

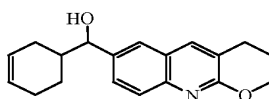


A mixture of intermediate 26 (0.0691 mol), 1-(phenylmethyl)- piperazine (0.0829 mol) and potassium carbonate (0.145 mol) in acetonitrile (150ml) was stirred and refluxed for 12 hours. The solvent was evaporated till dryness. The residue was taken up in DCM and water. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue (24.6g) was purified by column chromatography over silica gel (20-45 μm) (eluent: DCM/MeOH/NH₄OH 98/2/0.1). The pure fractions

were collected and the solvent was evaporated. The residue (2.7g) was crystallized from DIPE. The precipitate was filtered off and dried, yielding 1.6g of intermediate 27, melting point 78°C.

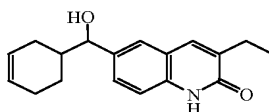
5 Example A7

a) Preparation of intermediate 28



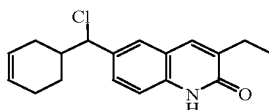
nBuLi 1.6M in hexane (0.224 mol) was added at -78°C under N₂ flow to a solution of 6-bromo-3-ethyl-2-methoxyquinoline (0.188 mol) in THF (500ml). The mixture was stirred at -78°C for 1 hour. A mixture of 3-cyclohexene-1-carboxaldehyde (0.182 mol) in THF (500ml) was added dropwise at -78°C. The mixture was stirred at -78°C for 2 hours, then brought to 0°C, hydrolyzed and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue (58.9g) was purified by column chromatography over silica gel (20-45 µm) (eluent: DCM/EtOAc 96/4). The pure fractions were collected and the solvent was evaporated, yielding 40.5g (72%) of intermediate 28.

b) Preparation of intermediate 29



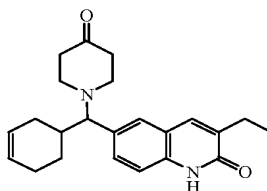
A mixture of intermediate 28 (0.131 mol) in hydrochloric acid 3N (400ml) and THF (400ml) was stirred at 60°C overnight and then basified with potassium carbonate solid. The precipitate was filtered off, washed with DCM and dried. The filtrate was extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was crystallized from DCM. The precipitate was filtered off and dried. Part (1.5g) of the residue (16.5g) was taken up in MeOH. The mixture was stirred overnight. The precipitate was filtered off and dried, yielding 0.72g of intermediate 29, melting point 212°C.

c) Preparation of intermediate 30



Intermediate 29 (0.053 mol) was added slowly at 0°C to thionyl chloride (150ml). The mixture was stirred at room temperature for 4 hours. The solvent was evaporated till dryness. The residue was taken up several times in DCM. The solvent was evaporated. The product was used without further purification, yielding 16g (quant.) of intermediate 30.

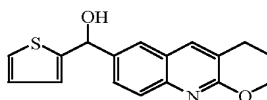
d) Preparation of intermediate 31



A solution of 4,4-piperidinediol, hydrochloride (0.079 mol) and potassium carbonate (0.265 mol) in DMF (200ml) was stirred at room temperature under N₂ flow for 10 min. A solution of intermediate 30 (0.053 mol) in DMF (200ml) was added slowly. The mixture was stirred at room temperature for 1 hour and then poured out into water. The precipitate was filtered off, washed several times with water and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue (19.2g) was purified by column chromatography over silica gel (15-40 μm) (eluent: DCM/MeOH/NH₄OH 96/4/0.2). The pure fractions were collected and the solvent was evaporated, yielding 11.4g (59%) of intermediate 31.

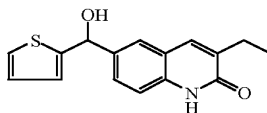
Example A8

a) Preparation of intermediate 32



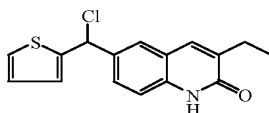
nBuLi 1.6M in hexane (0.154 mol) was added dropwise at -60°C under N₂ flow to a mixture of 6-bromo-3-ethyl-2-methoxy- quinoline (0.118 mol) in THF (314ml) and the mixture was stirred at -60°C for 1h. 2-thiophenecarboxaldehyde (0.142 mol) in THF (100ml) was added dropwise at -60°C. The mixture was stirred at -60°C for 2h, then at -40°C for 1h, poured out into a saturated NH₄Cl solution and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The product was used without further purification, yielding 35.37g (quant.) of intermediate 32.

b) Preparation of intermediate 33



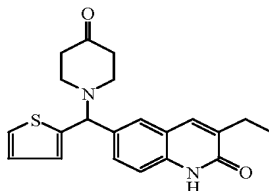
A mixture of intermediate 32 (0.118 mol) in hydrochloric acid 3N (426ml) and THF (274ml) was stirred at 70°C for 6 hours. The mixture was poured out on ice, basified with a concentrated NH₄OH solution and extracted with EtOAc. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (20-45 μm) (eluent: DCM/MeOH 98/2). The pure fractions were collected and the solvent was evaporated, yielding 9.3g (28%) of intermediate 33.

c) Preparation of intermediate 34



Intermediate 33 (0.0322 mol) was added portionwise at 0°C to thionyl chloride (46ml). The mixture was stirred at room temperature overnight. The solvent was evaporated till dryness. The product was used without further purification, yielding 9.78g (quant.) of intermediate 34.

d) Preparation of intermediate 35

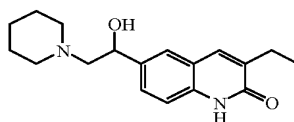


- 5 Potassium carbonate (0.161 mol) was added to a mixture of 4,4-piperidinediol, hydrochloride (0.0483 mol) in acetonitrile (74ml). The mixture was stirred under N₂ flow for 15 min. A mixture of intermediate 34 (0.0322 mol) in acetonitrile (98ml) was added at room temperature. The mixture was stirred at 60°C overnight, then poured out into water and extracted with DCM. The organic layer was separated, dried (MgSO₄),
- 10 filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15-40 μm) (eluent: DCM/MeOH/NH₄OH 97/3/0.1). The pure fractions were collected and the solvent was evaporated, yielding 0.46g (4%) of intermediate 35.

15 B. Preparation of the final compounds

Example B1

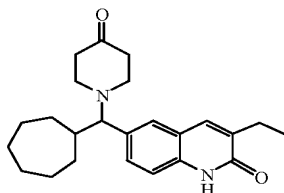
Preparation of compound 1



- Sodium hydroborate (0.0318 mol) was added at 0°C under N₂ flow to a solution of intermediate 3 (0.0245 mol) in MeOH (80ml). The mixture was stirred for 30 min. Water (10ml) was added. The organic solvent was evaporated. The aqueous concentrate
- 20 was taken up in DCM and water and the mixture was extracted. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated till dryness. Part (3g) of the residue (7.5g) was crystallized from 2-propanone and a small amount of MeOH. The precipitate was filtered off and dried, yielding 2.69g of compound 1, melting point 172°C.

Example B2

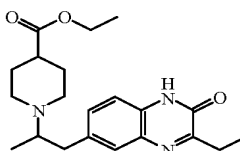
Preparation of compound 2



Potassium carbonate (0.107 mol) was added to a mixture of 4,4-piperidinediol hydrochloride (0.032 mol) in acetonitrile (49ml). The mixture was stirred under N₂ flow for 15 min. A mixture of intermediate 7 (0.0213 mol) in acetonitrile (68ml) was added. The mixture was stirred at 60°C for 3 hours, then poured out into water and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15-40 μm) (eluent: DCM/MeOH/NH₄OH 98.5/1.5/0.1). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from diethyl ether. The precipitate was filtered off and dried, yielding 4.16g (51%) of compound 2, melting point 218°C.

Example B3

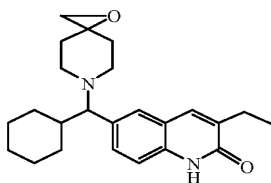
Preparation of compound 3



A mixture of intermediate 16 (0.002 mol) and ethyl 2-oxobutanoate (0.004 mol) in EtOH (15ml) was stirred and refluxed for 2.5 hours, poured out into water and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue (0.9g) was purified by column chromatography over silica gel (15-40 μm) (eluent: cyclohexane/2-propanol/NH₄OH 85/15/1). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from diethyl ether. The precipitate was filtered off and dried, yielding 0.054g of compound 3, melting point 163°C.

Example B4

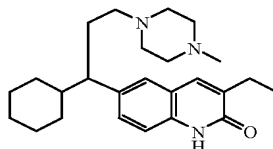
Preparation of compound 4



A mixture of sodium hydride (0.42g) in THF (10.5ml) was stirred at room temperature for 10 min. Then THF was decanted off. DMSO (32ml), then trimethylsulfoxonium iodide (0.013 mol) were added. The mixture was stirred at room temperature for 1 hour. Intermediate 20 (0.0114 mol) was added slowly. The mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was crystallized from 2-propanone/diethyl ether. The precipitate was filtered off and dried. The residue was recrystallized from 2-propanone/diethyl ether. The precipitate was filtered off and dried, yielding 1.54g (36%) of compound 4, melting point 200°C.

Example B5

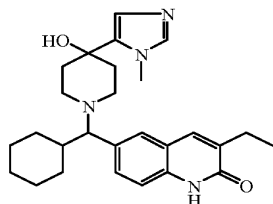
Preparation of compound 5



A mixture of intermediate 22 (0.00623 mol) in MeOH (25ml) was hydrogenated at room temperature under a 3 bar pressure for 8 hours with Pd/C 10% (0.25g) as a catalyst. After uptake of H₂ (1 equiv), the catalyst was filtered through celite and the filtrate was evaporated. The residue was taken up in water and a concentrated NH₄OH solution and the mixture was extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15-40 μm) (eluent: DCM/MeOH/NH₄OH 94/6/0.3). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from 2-propanone. The precipitate was filtered off and dried, yielding 1.07g (43%) of compound 5, melting point 181°C.

Example B6

Preparation of compound 6

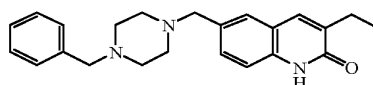


nBuLi 1.6M in hexane (21.32ml) was added dropwise at -70°C under N₂ flow to a mixture of 1-methyl- 1*H*-imidazole (0.0341 mol) in THF (28ml). The mixture was stirred at -70°C for 30 min. Chlorotriethyl- silane (0.0341 mol) was added. The mixture was brought to room temperature. nBuLi 1.6 M in hexane (21.32ml) was added

dropwise at -70°C . The mixture was stirred at -70°C for 1 hour and then brought to -15°C . A mixture of intermediate 20 (0.0136 mol) in THF (50ml) was added dropwise at -70°C . The mixture was allowed to warm to room temperature during the night, then poured out into a saturated NH_4Cl solution and extracted with EtOAc. The organic layer was separated, dried (MgSO_4), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (20-45 μm) (eluent: DCM/MeOH/ NH_4OH 94/6/0.5). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from 2-propanone. The precipitate was filtered off and dried, yielding 5.05g (83%) of compound 6, melting point 194°C .

Example B7

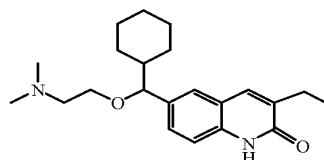
Preparation of compound 7



A mixture of intermediate 27 (0.00319 mol) in hydrochloric acid 6N (70ml) was stirred at 80°C for 30 min, poured out into water (50ml) and potassium carbonate solid. The mixture was stirred for 10 min. The precipitate was filtered off, rinsed with water and dried, yielding 0.9g (78%) of compound 7, melting point 194°C .

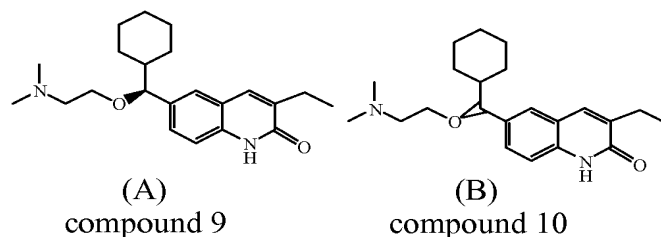
Example B8

a) Preparation of compound 8



A mixture of intermediate 19 (0.0164 mol) in 2-(dimethylamino)- ethanol (50ml) was stirred and refluxed for 2 hours. The mixture was poured out into water and extracted with DCM. The organic layer was separated, dried (MgSO_4), filtered and the solvent was evaporated. The residue (16g) was purified by column chromatography over silica gel (15-40 μm) (eluent: DCM/MeOH/ NH_4OH 94/6/0.5). The pure fractions were collected. The mixture was allowed to crystallize out for several days (precipitation resulted). The precipitate was filtered off, taken up in petroleum ether, filtered off and dried, yielding 2.8g (48%) of compound 8, melting point 122°C .

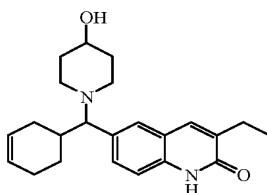
b) Preparation of compound 9 and 10



Compound 8 (0.02244 mol) was separated into its enantiomers by column chromatography (eluent: hexane/2-propanol 88/12; column: CHIRALPAK AD). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from hexane and petroleum ether. The precipitate was filtered off and dried, yielding 2.2g of compound 9, melting point 115°C, and 2.02g of compound 10, melting point 115°C.

Example B9

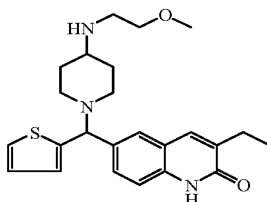
Preparation of compound 11



Sodium cyanotrihydroborate (0.02 mol) was added portionwise at 0°C under N₂ flow to a solution of intermediate 31 (0.02 mol) and 2-methoxy-ethanamine (0.024 mol) in MeOH (100ml). The mixture was stirred at room temperature for 12 hours. Water was added and the mixture was extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue (9.7g) was purified by column chromatography over silica gel (20-45 μm) (eluent: DCM/MeOH/NH₄OH 93/7/0.5). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from 2-propanone and diethyl ether. The precipitate was filtered off and dried, yielding 0.63g of compound 11, melting point 196°C.

Example B10

Preparation of compound 12

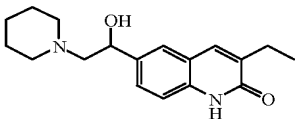
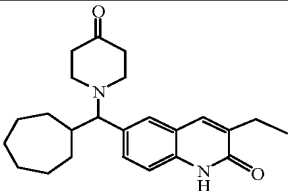
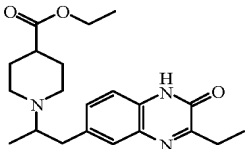
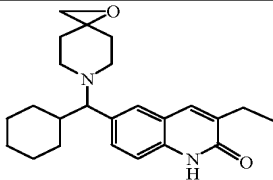
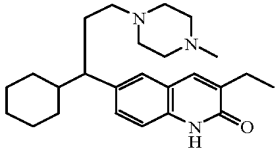
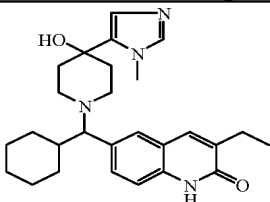
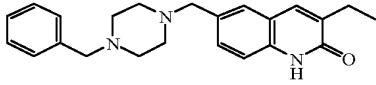
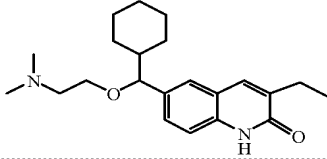
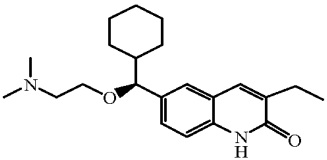
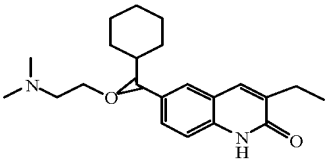


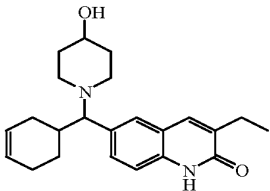
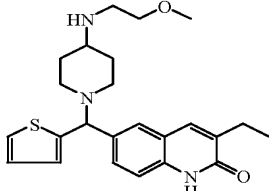
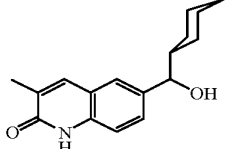
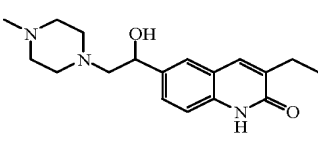
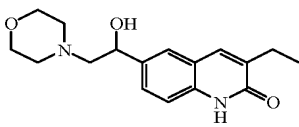
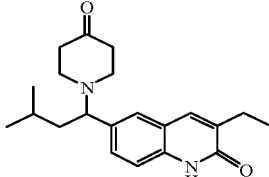
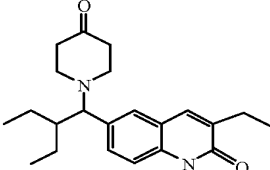
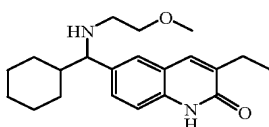
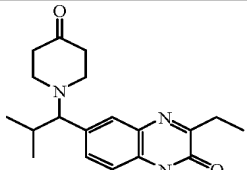
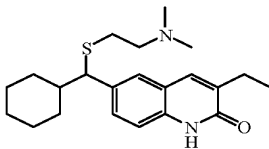
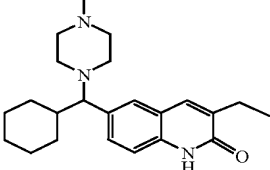
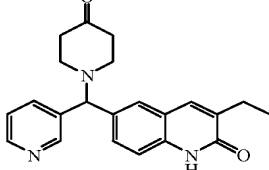
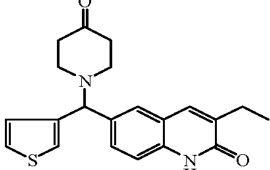
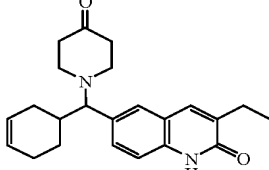
Sodium cyanotrihydroborate (0.00126 mol) was added at 0°C to a mixture of intermediate 35 (0.00126 mol) and 2-methoxy-ethanamine (0.00151 mol) in MeOH (10ml). The mixture was stirred at room temperature overnight, then poured out on ice

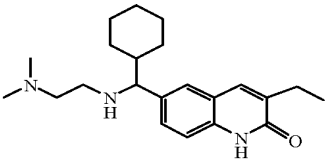
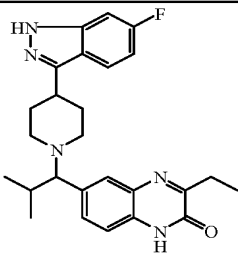
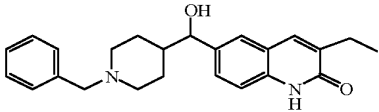
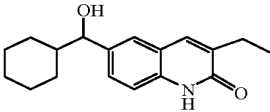
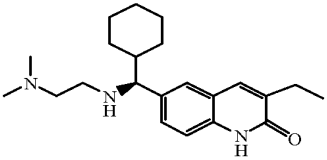
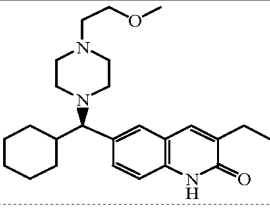
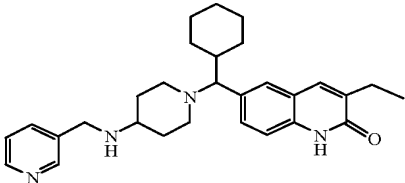
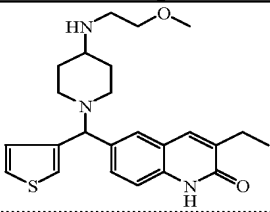
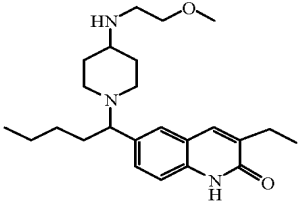
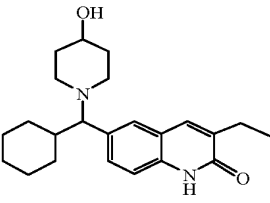
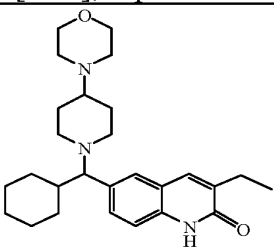
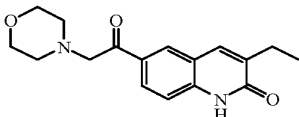
and extracted with DCM. The organic layer was separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15-40 μm) (eluent: DCM/MeOH/NH₄OH 90/10/0.1). The pure fractions were collected and the solvent was evaporated. The residue was crystallized from MEK and diethyl ether. The precipitate was filtered off and dried, yielding 0.22g (41%) of compound 12.

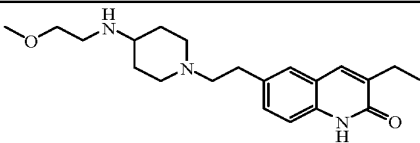
Table F-1 lists the compounds that were prepared according to one of the above Examples.

Table F-1

	
Co. No. 1; Ex. [B1]; mp. 172°C	Co. No. 2; Ex. [B2]; mp. 218°C
	
Co. No. 3; Ex. [B3]; mp. 163°C	Co. No. 4; Ex. [B4]; mp. 200°C
	
Co. No. 5; Ex. [B5]; mp. 181°C	Co. No. 6; Ex. [B6]; mp. 194°C
	
Co. No. 7; Ex. [B7]; mp. 194°C	Co. No. 8; Ex. [B8]; mp. 115°C
	
(A); Co. No. 9; Ex. [B8]; mp. 115°C	(B); Co. No. 10; Ex. [B8]; mp. 115°C

	
<p>Co. No. 11; Ex. [B9]; mp. 196°C</p>	<p>Co. No. 12; Ex. [B10]; mp. 148°C</p>
	
<p>Co. No. 13; Ex. [B1]; mp. 242.2°C</p>	<p>Co. No. 14; Ex. [B1]; mp. 176°C</p>
	
<p>Co. No. 15; Ex. [B1]; mp. 175°C</p>	<p>Co. No. 16; Ex. [B2]; mp. 180°C</p>
	
<p>Co. No. 17; Ex. [B2]; mp. 181°C</p>	<p>Co. No. 18; Ex. [B2]; mp. 100°C</p>
	
<p>Co. No. 19; Ex. [B2]; mp. 136°C</p>	<p>Co. No. 20; Ex. [B2]; mp. 126°C</p>
	
<p>Co. No. 21; Ex. [B2]; mp. 228°C</p>	<p>Co. No. 22; Ex. [B2]; mp. 184°C</p>
	
<p>Co. No. 23; Ex. [B2]; mp. 213°C</p>	<p>Co. No. 24; Ex. [B2]; mp. 203°C</p>

	
<p>Co. No. 25; Ex. [B2]; mp. 154°C</p>	<p>Co. No. 26; Ex. [B2]; mp. 152°C</p>
	
<p>Co. No. 27; Ex. [B7]; mp. 193°C</p>	<p>Co. No. 28; Ex. [B7]; mp. 230°C</p>
	
<p>(A); Co. No. 29; Ex. [B8]; mp. 106°C</p>	<p>(A); .C₂H₂O₄ (1:2). H₂O (1:1); Co. No. 30; Ex. [B8]; mp. 196°C</p>
	
<p>Co. No. 31; Ex. [B10]</p>	<p>Co. No. 32; Ex. [B10]; mp. 144°C</p>
	
<p>.C₂H₂O₄ (1:2) .H₂O (1:1); Co. No. 33; Ex. [B10]; mp. 235°C</p>	<p>Co. No. 34; Ex. [B10]; mp. 198°C</p>
	
<p>Co. No. 35; Ex. ; mp. 222°C</p>	<p>Co. No. 36; Ex. [B10]; mp. 216°C</p>

	
Co. No. 37; Ex. [B10]; mp. 132°C	

Pharmacological example

In vitro Scintillation Proximity Assay (SPA) for PARP-1 inhibitory activity

- 5 Compounds of the present invention were tested in an *in vitro* assay based on SPA technology (proprietary to Amersham Pharmacia Biotech).

In principle, the assay relies upon the well established SPA technology for the detection of poly(ADP-ribosyl)ation of biotinylated target proteins, i.e histones. This ribosylation is induced using nicked DNA activated PARP-1 enzyme and [³H]-

- 10 nicotinamide adenine dinucleotide ([³H]-NAD⁺) as ADP-ribosyl donor.

As inducer of PARP-1 enzyme activity, nicked DNA was prepared. For this, 25 mg of DNA (supplier: Sigma) was dissolved in 25 ml DNase buffer (10 mM Tris-HCl, pH 7.4; 0.5 mg/ml Bovine Serum Albumine (BSA); 5 mM MgCl₂·6H₂O and 1 mM KCl) to which 50 µl DNase solution (1mg/ml in 0.15 M NaCl) was added. After an incubation of 90 min. at 37 °C, the reaction was terminated by adding 1.45 g NaCl, followed by a further incubation at 58 °C for 15 min. The reaction mixture was cooled on ice and dialysed at 4 °C for respectively 1.5 and 2 hours against 1.5 l of 0.2 M KCl, and twice against 1.5 l of 0.01 M KCl for 1.5 and 2 h respectively. The mixture was aliquoted and stored at –20 °C. Histones (1 mg/ml, type II-A, supplier: Sigma) were biotinylated using the biotinylation kit of Amersham and stored aliquoted at – 20 °C. A stock solution of 100 mg/ml SPA poly(vinyl toluene) (PVT) beads (supplier: Amersham) was made in PBS. A stock solution of [³H]-NAD⁺ was made by adding 120 µl of [³H]-NAD⁺ (0.1 mCi/ml, supplier: NEN) to 6 ml incubation buffer (50 mM Tris/HCl, pH 8; 0.2 mM DTT; 4 mM MgCl₂). A solution of 4 mM NAD⁺ (supplier: Roche) was made in incubation buffer (from a 100 mM stock solution in water stored at – 20 °C). The PARP-1 enzyme was produced using art known techniques, i.e. cloning and expression of the protein starting from human liver cDNA. Information concerning the used protein sequence of the PARP-1 enzyme including literature references can be found in the Swiss-Prot database under primary accession number P09874. Biotinylated histones and PVT-SPA beads were mixed and pre-incubated for 30 min. at room temperature. PARP-1 enzyme (concentration was lot dependent) was mixed with the nicked DNA and the mixture was pre-incubated for 30 min. at 4 °C. Equal parts of this

histones/PVT-SPA beads solution and PARP-1 enzyme/DNA solution were mixed and 75 μ l of this mixture together with 1 μ l of compound in DMSO and 25 μ l of [3 H]-NAD $^+$ was added per well into a 96-well microtiterplate. The final concentrations in the incubation mixture were 2 μ g/ml for the biotinylated histones, 2 mg/ml for the PVT-SPA beads, 2 μ g/ml for the nicked DNA and between 5 – 10 μ g/ml for the PARP-1 enzyme. After incubation of the mixture for 15 min. at room temperature, the reaction was terminated by adding 100 μ l of 4 mM NAD $^+$ in incubation buffer (final concentration 2 mM) and plates were mixed.

The beads were allowed to sediment for at least 15 min. and plates transferred to a TopCountNXT $^{\text{TM}}$ (Packard) for scintillation counting, values were expressed as counts per minute (cpm). For each experiment, controls (containing PARP-1 enzyme and DMSO without compound), a blank incubation (containing DMSO but no PARP-1 enzyme or compound) and samples (containing PARP-1 enzyme and compound dissolved in DMSO) were run in parallel. All compounds tested were dissolved and eventually further diluted in DMSO. In first instance, compounds were tested at a concentration of 10^{-6} M. When the compounds showed activity at 10^{-6} M, a dose-response curve was made wherein the compounds were tested at concentrations between 10^{-5} M and 10^{-8} M. In each test, the blank value was subtracted from both the control and the sample values. The control sample represented maximal PARP-1 enzyme activity. For each sample, the amount of cpm was expressed as a percentage of the mean cpm value of the controls. When appropriate, IC $_{50}$ -values (concentration of the drug, needed to reduce the PARP-1 enzyme activity to 50% of the control) were computed using linear interpolation between the experimental points just above and below the 50 % level. Herein the effects of test compounds are expressed as pIC $_{50}$ (the negative log value of the IC $_{50}$ -value). As a reference compound, 4-amino-1,8-naphthalimide was included to validate the SPA assay. The tested compounds showed inhibitory activity at the initial test concentration of 10^{-6} M (see Tabel-2).

In vitro filtration assay for PARP-1 inhibitory activity

Compounds of the present invention were tested in an *in vitro* filtration assay assessing PARP-1 activity (triggered in the presence of nicked DNA) by means of its histone poly (ADP-ribosyl)ation activity using [32 P]-NAD as ADP-ribosyl donor. The radioactive ribosylated histones were precipitated by trichloroacetic acid (TCA) in 96-well filterplates and the incorporated [32 P] measured using a scintillation counter

A mixture of histones (stock solution: 5 mg/ml in H₂O), NAD⁺ (stock solution: 100 mM in H₂O), and [³²P]-NAD⁺ in incubation buffer (50 mM Tris/HCl, pH 8; 0.2 mM DTT; 4 mM MgCl₂) was made. A mixture of the PARP-1 enzyme (5 – 10 µg/ml) and nicked DNA was also made. The nicked DNA was prepared as described in the *in vitro* SPA for PARP-1 inhibitory activity. Seventy-five µl of the PARP-1 enzyme/DNA mixture together with 1 µl of compound in DMSO and 25 µl of histones-NAD⁺/[³²P]-NAD⁺ mixture was added per well of a 96-well filterplate (0.45 µm, supplier Millipore). The final concentrations in the incubation mixture were 2 µg/ml for the histones, 0.1 mM for the NAD⁺, 200 µM (0.5 µCi) for the [³²P]-NAD⁺ and 2 µg/ml for the nicked DNA. Plates were incubated for 15 min. at room temperature and the reaction was terminated by the addition of 10 µl ice cold 100% TCA followed by the addition of 10 µl ice-cold BSA solution (1 % in H₂O). The protein fraction was allowed to precipitate for 10 min. at 4 °C and plates were vacuum filtered. The plates were subsequently washed with, for each well, 1 ml of 10 % ice cold TCA, 1 ml of 5 % ice cold TCA and 1 ml of 5 % TCA at room temperature. Finally 100 µl of scintillation solution (Microscint 40, Packard) was added to each well and the plates were transferred to a TopCountNXTTM (supplier: Packard) for scintillation counting and values were expressed as counts per minute (cpm). For each experiment, controls (containing PARP-1 enzyme and DMSO without compound), a blank incubation (containing DMSO but no PARP-1 enzyme or compound) and samples (containing PARP-1 enzyme and compound dissolved in DMSO) were run in parallel. All compounds tested were dissolved and eventually further diluted in DMSO. In first instance, compounds were tested at a concentration of 10⁻⁵M. When the compounds showed activity at 10⁻⁵M, a dose-response curve was made wherein the compounds were tested at concentrations between 10⁻⁵M and 10⁻⁸M. In each test, the blank value was subtracted from both the control and the sample values. The control sample represented maximal PARP-1 enzyme activity. For each sample, the amount of cpm was expressed as a percentage of the mean cpm value of the controls. When appropriate, IC₅₀-values (concentration of the drug, needed to reduce the PARP-1 enzyme activity to 50% of the control) were computed using linear interpolation between the experimental points just above and below the 50 % level. Herein the effects of test compounds are expressed as pIC₅₀ (the negative log value of the IC₅₀-value). As a reference compound, 4-amino-1,8-naphthalimide was included to validate the filtration assay. The tested compounds showed inhibitory activity at the initial test concentration of 10⁻⁵M (see Tabel-2).

Tabel-2

Co.No.	In vitro SPA	In vitro filtration assay
	pIC50	pIC50
1	6.483	6.457
2	6.634	6.246
3	6.065	
4	6.523	5.817
5	6.295	5.296
6	6.294	5.87
7	7.024	
8	6.059	5.172
9	6.212	5.359
10	< 6	
11	6.735	6.105
12	6.726	5.644
14	6.411	5.735
15	6.54	5.595
16	6.639	5.735
17	6.417	5.592
18	6.125	5.524
19	6.121	
20	6.222	5.735
21	6.045	
22	6.703	5.928
23	6.634	5.898
24	6.557	5.875
25	5.956	5.221
26	6.271	
27	6.744	5.657
28	6.54	5.502
29	6.075	5.266
30	5.894	5.037
31	6.064	5.083
32	6.707	5.548
33	6.08	5.376
34	6.069	5.615
35	6.194	5.468
36	6.246	5.584
37	6.069	

5 The compounds can be further evaluated in a cellular chemo- and/or radiosensitization assay, an assay measuring inhibition of endogenous PARP-1 activity in cancer cell lines and eventually in an *in vivo* radiosensitization test.